CARBOHYDRATE METABOLISM

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CORRELATION OF PHYSIOLOGICAL, BIOCHEMICAL AND CLINICAL ASPECTS

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PREFACE

THIS volume is intended to serve as a correlative text for the teaching of carbohydrate metabolism to students of physiology, biochemistry, and medicine If the authors have succeeded in their endeavor, they will have satisfied a hitherto unmet need in this field The various aspects of carbohydrate metabolism usually have been taught as separate subjects by the different departments of universities and medical schools This can hardly be avoided under the present system of teaching organization, but the arrangement has obvious disadvantages Not uncommonly the net result for the student is a disjointed, incomplete, and often contradictory presentation of the subject as a whole It is the hope of the authors that the use of this text as a common meeting ground by the appropriate departments of the same institution will be of help to both student and teacher

A fortunate corollary of this integration of the subject is that it should make the volume useful to the practicing physician who seeks to keep abreast of the fundamentals upon which his clinical applications are based The material is not otherwise available except in an extensive and highly technical periodical literature, with which he cannot be expected to cope directly This applies particularly to the newer knowledge of tissue enzyme chemistry and to the pathological physiology of diabetes, a subject which has undergone a revolutionary development within the past few decades

Despite its title, this volume also deals in considerable detail with certain asaspects of protein and fat metabolism This is mentioned to emphasize the increasingly obvious fact that the traditional didactic separation between the metabolisms of the three chief foodstuffs is largely artificial Those restrictions which the present authors have placed on the scope of the subject matter depend more upon their own limitations than upon any real division of the material

The more than twelve hundred references cited by no means represent a complete bibliography of the subject They have been carefully selected as original sources of crucial experimental facts or because they review certain aspects of the subject in greater detail than is feasible in this text or because they contain useful references to the many good scientific articles which could not even be mentioned in the present volume

The senior author wishes to acknowledge the major contributions of his associates, past and present, to the development of the concepts discussed in this book

He also wishes to express his gratitude to the Michael Reese Hospital for the ample support and academic freedom granted him to the University of Chicago for the teaching and intellectual associations which he has been privileged to enjoy and to the Committee on Publications in Biology and Medicine of the University for the stimulation without which this book might not have been undertaken

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PART I

THE BIOCHEMISTRY AND ENERGETICS OF CARBOHYDRATE METABOLISM

CHAPTER 1

THE IMPORTANCE OF CARBOHYDRATES IN NUTRITION

THE importance of carbohydrates in human nutrition has varied greatly at different times and in different parts of the world Grains, fruits, and vegetables are the natural foods which are high in carbohydrate content Meat, fish, and dairy products are relatively poor in this constituent Before the development of the modern food processing and distributing industry (and, at the present time, in those parts of the world which have not undergone this develop ment) the proportion of carbohydrate in the diet of any region was largely gov erned by the local flora and fauna Thus, even now the proportionate intake of carbohydrate is high in tropical countries, where vegetation is luxurious and where the climate leads to rapid spoilage of meat products For the obverse reasons, the inhabitants of the Far North have always lived on a diet which consists chiefly of meat and fish Adequate nutrition is possible at both extremes of this range of dietary variation, provided that the need for calories essential food factors, vita mins and minerals is met (1, 2 3, 4)

Although there has been some change during the last fifty years in the food sources from which the carbohydrates are derived, the proportion of carbohydrate in the dietary of the United States has remained at about 50–60 per cent of the total caloric intake Since certain foods which are high in carbohydrate content are relatively inexpensive, the proportion of carbohydrate in the diet has been greater at lower economic levels than in the more prosperous groups of the popu lation However, the poorer nutritional status of the lowest income groups is not so much a reflection of their high carbohydrate intake as it is a result of the par ticular foods from which they derive their carbohydrates The highly refined grains and sugars, which have been commercially developed largely because of their re sistance to spoilage are the cheapest sources of calories generally available But they have coincidentally been deprived of most of the protective elements with which they are naturally associated, so that a *casually selected* high carbohydrate diet is likely to be poor in the essential amino acids, vitamins, and minerals (5)

THE CARBOHYDRATES IN FOOD

The particular carbohydrates present in the ordinary American diet, the food sources from which these carbohydrates are derived, and the quantitative impor- tance of each carbohydrate in the total intake are indicated in Table 1

TABLE 1

TYPES AND SOURCES OF CARBOHYDRATES IN THE AMERICAN DIETARY (6)

Carbohydrates	Approximate Percentage of Total Carbohydrate Intake*	Chief Food Sources	End Products of Digestion	Remarks
Polysaccharides				
a) Indigestible	3			
1 Celluloses and hemicelluloses		Stalks and leaves of vegetables, outer covering of seeds	0	May be partially split to glucose by bacterial action in large bowel
2 Pectins		Fruits	0	Chemical hydrolysis yields galactose and arabinose
b) Partially digestible	2			
1 Inulin		Jerusalem artichokes, onions, garlic	Fructose	
2 *Galactogens*		Snails	Galactose	
3 Mannosans		Legumes	*Mannose*	
4 Raffinose		Sugar beets	Glucose, fructose, and galactose	*Digestion incomplete*, further splitting by bacteria may occur in large bowel
5 *Pentosans*		Fruits and gums	Pentoses	
c) Digestible				
1 Starch and dextrins	50	Grains, vegetables (especially tubers and legumes)	Glucose	The most important group quantitatively Usually accompanied by some maltose
2 Glycogen	Negligible	Meat products and sea food	Glucose	
Disaccharides				
1 Sucrose	25	Cane and beet sugars, molasses, *maple syrup*	Glucose and fructose	
2 Lactose	10	Milk and milk products	Glucose and galactose	
3 Maltose	Negligible	Malt products	Glucose	

*Calculated from the average dietary of the middle-income group in the United States

TABLE 1—*Continued*

Carbohydrates	Approximate Percentage of Total Carbohydrate Intake	Ch ef Food Sources	End-Products of Digestion	Remarks
Monosaccharides				
a) Hexoses				
1 Glucose	5	Fruits honey corn syrup	Glucose	In fruits and vegetables the contents of glucose and fructose depend on species, ripeness and state of preservation
2 Fructose	5	Fruits, honey	Fructose	
3 Galactose	o	o	Galactose	These monosaccharides do not occur in free form in foods see under lactose and mannosans
4 Mannose	o	o	Mannose	
b) Pentoses				
1 Ribose	o	o	Ribose	These monosaccharides do not occur in free form in foods They are derived from pentosans of fruits and from the nucleic acids of meat products and sea food
2 Xylose	o	o	Xylose	
3 Arabinose	o	o	Arabinose	
rbohydrate derivatives				
1 Ethyl alcohol	Variable	Fermented liquors	Absorbed as such	These substances are the products of natural or induced carbohydrate breakdown
2 Lactic acid	Negligible	Milk and milk products		
3 Malic acid	Negligible	Fruits		
4 Citric acid	Negligible	Fruits		

THE DIGESTION OF CARBOHYDRATES (7)

The digestion of carbohydrates starts in the oral cavity Here the secretion of the parotid gland, which contains an amylase called "ptyalin," is mixed with the food and begins the conversion of starch, glycogen, and the dextrins into maltose This digestion continues in the stomach until the hydrochloric acid which is secreted there destroys the amylase activity and substitutes acid hydrolysis for enzymatic splitting If continued long enough, the acid hydrolysis can reduce all the digestible carbohydrates to the monosaccharide stage However, the stomach usually empties itself before this can occur, and the digestion of carbohydrate is taken up by the enzymes of the small intestine, operating in the more alkaline medium which prevails there The enzymes in the small intestine are an amylase secreted by the pancreas, and an amylase, a maltase, an invertase, and a lactase secreted by the wall of the small bowel All these enzymes are capable of splitting the particular sugars which they attack to the monosaccharide stage

We have accounted for the digestion of starch, glycogen, the dextrins, and the disaccharides Those sugars which are ingested in the form of monosaccharides do not require digestion All the remaining carbohydrates pass through the stomach and small intestine unchanged In the large bowel they are subjected to the enzymatic influence of the profuse bacterial flora which is normal there, and they may be broken down to monosaccharides to some extent It is possible that minor amounts of carbohydrate are made available in this manner for absorption into the blood stream (see Fig 1)

THE ABSORPTION OF CARBOHYDRATES

The monosaccharides, ingested as such or arising from the digestion of carbohydrates, are practically completely absorbed in the small intestine Small amounts may be absorbed from the stomach It is also possible to show that, *when solutions of monosaccharides are introduced into the large bowel for experimental or therapeutic purposes*, some sugar can be absorbed from this portion of the gastrointestinal tract (8, 9)

Two types of absorption occur in the small intestine (*a*) a specific absorption of particular monosaccharides, probably involving a phosphorylation process, and (*b*) a non specific absorption of all monosaccharides, by diffusion resulting from osmotic forces across the mucous membrane (10, 11) Glucose, fructose, and galactose are absorbed by both processes Consequently, the absorption of these sugars differs in two respects from that of those sugars that are absorbed by diffusion alone they are absorbed more rapidly, and their rates of absorption are largely independent of their concentrations in the intestine (12) The explanation for the greater efficiency of specific absorption is apparently the coupling of the monosaccharide with phosphate as soon as it diffuses into the wall of the intestine This phosphorylation is a rapid process, so that the gradient of the concentration

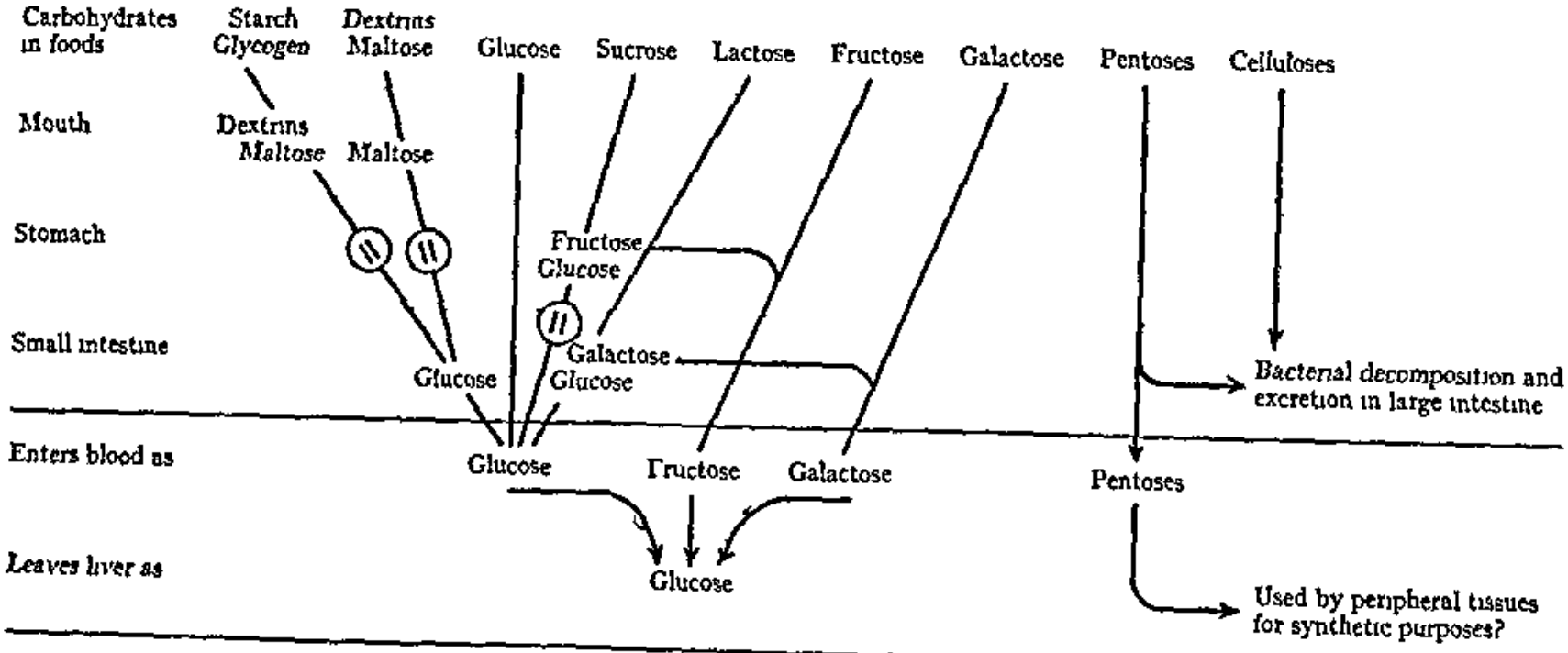

Fig. 2.—Products of carbohydrate digestion at various levels of the gastro-intestinal tract, and subsequent fate. ⑪ indicates that the same products as at the preceding level continue to appear

of free sugar between the lumen and the wall of the gut is much steeper than when absorption proceeds by diffusion alone

The actual rates of absorption of the three monosaccharides which are phosphorylated vary rather widely, though all are much higher than the absorption rates of such monosaccharides as mannose or the pentoses, which are handled by diffusion Thus it has been shown in rats that, if the rate for glucose is represented as 100, that for galactose would be 110, for fructose 43, and for mannose and the pentoses only 9 (13) There are few reliable data on the absolute rates at which the various monosaccharides can be absorbed from the gastro intestinal tract of the human being under normal circumstances The best available evidence from the work of Groen (14) indicates that the rate of absorption of glucose from a 50 cm length of jejunum (small intestine) is about 8 0 gm per hour, that for galactose, about 9 5 gm per hour, and that for fructose, about 5 gm per hour These rates are for concentrations of sugar of 10 per cent and above Below 10 per cent the rate of absorption varies directly with the concentration

From the practical standpoint the figures quoted above may have little relationship to the rate at which a monosaccharide enters the blood stream, whether eaten as such or arising from the processes of digestion under the usual conditions of feeding Under the latter circumstances the time which elapses before it is absorbed from the gastro intestinal tract will be governed largely by (*a*) the rate at which it enters the small intestine and (*b*) the mixture of foods in the small intestine at the time of absorption The rate at which the sugar arrives at the small intestine depends largely on the motility of the stomach and the control of the pyloric sphincter, which can be affected by such various phenomena as hunger, emotion, local irritation (including condiments), and the composition and consistency of the food mass after mastication (15) The food mixture in the small intestine affects the rate of absorption by competition of the various constituents in the mixture for the absorbing surface of the mucosa and, in the case of those monosaccharides which are specifically absorbed, by competition for the available phosphorylating capacity (15)

Other factors which influence the amount of carbohydrate absorbed in a given individual at a particular time are (*a*) the normality of the mucous membrane of the small intestine and the length of time during which the carbohydrate is in contact with it, (*b*) endocrine function, particularly that of the anterior pituitary gland (16), the thyroid (17), and the adrenal cortex (18), and (*c*) the adequacy of vitamin intake, especially that of the B complex (19, 20, 21) Since the absorption of the important end products of carbohydrate digestion requires chemical activity by the mucous membrane, it is obvious that any abnormality of the mucosal cells might interfere with carbohydrate absorption Enteritis (inflammation) is a not uncommon disturbance of this kind Coeliac disease (22) may represent a more obscure disturbance of a similar nature However, even when the mucosa is

normal, an excessive rate of movement of the carbohydrate along the gastro intestinal tract, accompanying diarrheas of various origins, may hurry a portion of the ingested carbohydrate into the large bowel before it can be absorbed

Normal absorption of carbohydrate does not occur in the presence of an anterior pituitary deficiency This probably depends, for the most part, upon the secondary hypofunction of the thyroid gland, for the same result may be obtained after removal of the thyroid gland when the hypophysis is intact Furthermore, the defect in absorption accompanying hypopituitarism may be relieved by the administration of thyroid extract (16) Indeed, Althausen and co workers (17, 23) have attempted to make use of this phenomenon as a clinical test of the state of activity of the thyroid gland They administer a standard amount of galactose by mouth, follow the rise of galactose concentration in the blood, and use the rate of the latter as a criterion of thyroid function

The adrenal cortex influences carbohydrate absorption through its regulation of the sodium chloride (NaCl) exchange in the body The absorption of carbohydrate from the intestine is subnormal in adrenal cortical deficiency but can be restored to normal without the use of adrenal cortical extracts if the NaCl of the blood is raised to normal levels by adequate salt intake (18)

Insulin, which has such an important influence on other aspects of carbohydrate metabolism, is without apparent effect upon the absorptive capacity of the intestinal mucous membrane

Deficiency of the B complex is associated with diminished absorption of the hexoses (19) Recent work on this subject has been concerned with the separate effects of the various pure components of the complex Thiamine, pantothenic acid, and pyridoxine affect absorption Riboflavin is without action (20, 21)

THE DISTRIBUTION OF CARBOHYDRATE IN THE BODY ITS FUNCTIONS AND USES

In order to understand the distribution of carbohydrate in the body and appreciate its particular functions and uses, it is necessary first to consider the relation of carbohydrate metabolism to that of the other two major foodstuffs

Protein constitutes 75 per cent of the dry weight of the soft tissues of the body (24) In view of the recent knowledge as to the protein nature of the tissue enzymes, it is a fair generalization to say that the proteins, together with the hormones, vitamins, and minerals, constitute the metabolic machinery of the body In emergencies a certain amount of the protein machinery can be broken down and converted into fuel [1] However, the amount of body protein which is available for this purpose at any one time is strictly limited *as is also the length of survival*

during exercise indicates that it is of secondary importance, probably to supply carbohydrate or carbohydrate intermediates The results of experiments on fat utilization during muscular work have demonstrated that this substance is used indirectly There is no experimental evidence at the present time for the direct utilization of fat by mammalian muscle However, the indirect utilization of protein or fat must be an efficient process since the exclusive feeding of these substances to man does not have a marked effect on muscular efficiency during short periods of exercise

The significance of the foregoing from the standpoint of nutrition is obvious If carbohydrate is not available in foods, it must be made by the body from those materials which are in the diet, in order to satisfy the fuel requirements of the active tissues The eating of adequate amounts of carbohydrate therefore spares the body the work of making its fuel This role of carbohydrate is naturally more

TABLE 3

DISTRIBUTION OF CARBOHYDRATE IN VARIOUS TISSUES OF RAT, DOG AND MAN
(Figures Represent Ranges Found on a Mixed Diet)

Tissue	Rat		Dog		Man	
	Glycogen (Per Cent)	Glucose (Mg per Cent)	Glycogen (Per Cent)	Glucose (Mg per Cent)	Glycogen (Per Cent)	Glucose (Mg per Cent)
Skeletal muscle	0 81 1 06 (33)*	50–70	0 55 (35)	40–60	0 4–0 6 (36)	
Liver	2 5 –8 3 (33)		6 10 (35)		1 5–6 0 (37)	
Heart	0 3 –0 6 (33)		0 47 (35)			
Kidney			0 15 (35)		0 4 (37)	
Brain	0 08 (34)		0 1 (34)	57 (34)		
Skin	0 07 (39)	77 (39)	0 08 (38)	71 (38)	0 08 (38)	60–82 (38)
Blood and extra cellular fluids		90–129 (33)		60–80		60–90

* Figures in parentheses refer to bibliographical references at end of chapter

important during moderate or severe muscular exertion than when the body is at rest The great demand for fuel accompanying muscular exercise may rapidly exhaust the carbohydrate stores This is evidenced by a decrease in glycogen content of the liver and muscles and, if the exertion is sufficiently severe and prolonged, may result in an abnormal lowering of the blood sugar level (41) These phenomena are accompanied by increased breakdown of body protein (which is reflected in an increased excretion of nitrogen in the urine [40]) and by an accelerated breakdown of body fat (as evidenced by a rise of the level of ketone bodies in blood and urine [42]) When violent exercise is preceded or accompanied by a large intake of carbohydrate, the body works somewhat more efficiently, as judged by the calories expended per unit of oxygen intake The increased nitrogen excretion and ketone formation are also minimized The latter two effects of carbohydrate are examples of its protein sparing and its antiketogenic actions

The efficiency of carbohydrate as a fuel —It has been noted above that carbohydrate is a more efficient fuel for muscular exercise than either protein or fat This does not imply that portions of the protein or fat molecules are wasted when they

are used It does mean that the protein and fat molecules, when used as fuel, yield less than their total caloric value in the form which can be used by muscle The remainder is used for the conversion of these molecules into suitable fuel These conversions occur largely in the liver, which supplies the other organs with fuel by way of the blood stream

Since the amount of glycogen present in the muscle at any one time is sufficient for only short periods of work, the carbohydrate used by the muscle must eventually come from the blood sugar The glycogen within the muscle cells may be reasonably supposed to serve best in emergencies, when the muscle is unable to draw sugar from the blood as quickly as needed But, as a matter of fact, glycogen is more than merely a conveniently packaged form of carbohydrate lying on the

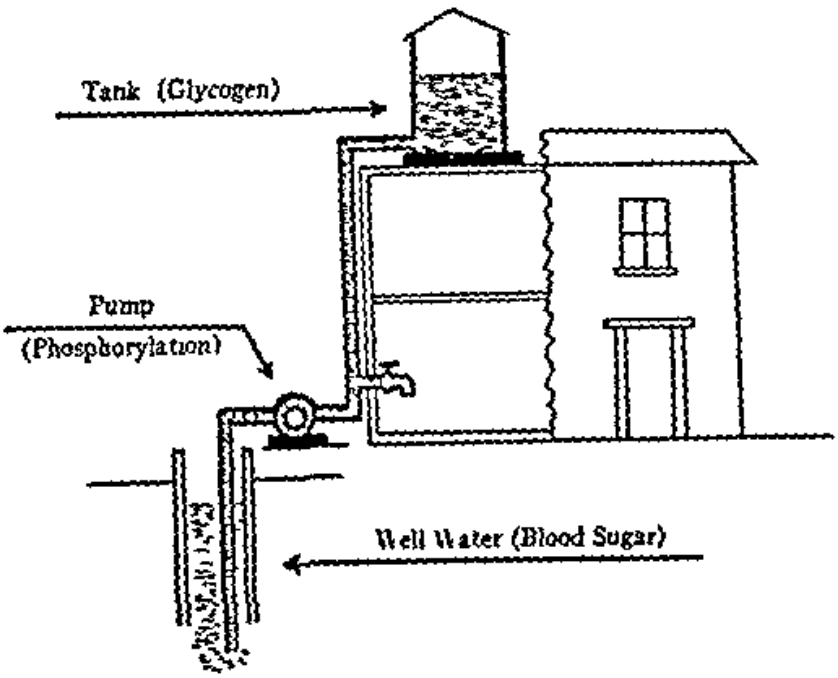

FIG 2.—Mechanical analogy illustrating the advantage of tissue glycogen over blood sugar as an emergency fuel. (Soskin [44])

pantry shelf It is now known that more energy is derivable from a certain amount of glycogen than from an equivalent amount of blood sugar It requires a certain amount of energy to bring the blood sugar into the metabolic system of the muscle (as hexose-6 phosphate [43]), and therefore all the energy inherent in the glucose is not available for useful work On the other hand, the breakdown of glycogen to the same stage does not require the addition of energy and hence makes all its inherent energy quickly available (43) This is not to say that one gets something for nothing from glycogen, for some energy was required to build up the glycogen in the first place But this energy was expended during a quiescent period when plenty of it was available

The above situation is analogous to that portrayed in Figure 2 (44) Here the water in the well represents the blood sugar, the pump stands for the phosphory-

lating mechanisms, and the tank on the roof represents the glycogen store It is readily understandable that, when the tank contains stored water, the tap can deliver a rate of flow far beyond the rate capacity of the pump The water stored during periods when the tap is closed is at a higher level than the original source of the water and also stores some of the energy applied by the pump This potential energy is released when the tap is opened Too great an outflow from the tap may, of course, exhaust the stored water and reduce the flow from the tap to the rate at which the pump is capable of operating A similar situation may occur in muscle when excessive rates of work over prolonged periods are attempted

The application of these physiological facts to clinical phenomena is exemplified by the greater stores of glycogen and of phosphate esters found in the muscles of animals which have been trained to perform prolonged work (45) This probably also applies to the physical abilities of manual laborers and of athletes Conversely, the characteristically low muscle glycogen levels found in poorly controlled diabetic patients and in hyperthyroid individuals are accompanied by muscular weakness

Special functions of carbohydrate in the liver —Aside from its use as fuel in the liver, carbohydrate in this organ has protective and detoxifying actions and a regu*lating influence on protein and fat metabolism*

The liver of a well fed normal animal contains a high percentage of glycogen, as compared to any other tissue It is known that such a liver is more resistant to various types of noxious agents than one which has been deprived of its glycogen by starvation or disease This has been shown in animals for such various types of poisons as carbon tetrachloride (46), alcohol (47), or arsenic (48) and in man for a variety of diseases accompanied by toxemias of bacterial origin (49, 50) The defenses of the liver against toxic agents are of great importance to the body as a whole, for it is one of the chief functions of this organ to remove or destroy such toxins before they reach other vital tissues which are not equipped to deal with them From this point of view, the maintenance of a high glycogen level in the liver is an essential for the health of the whole organism

It is now known that most of the glycogen of the liver is present in the form of a complex with protein (51) It is a reasonable assumption that, just as the protein part of the complex stabilizes the glycogen, so the glycogen would tend to protect the protein More definite knowledge is available as regards the role of carbohydrate in specific chemical reactions which transform certain poisons into relatively innocuous substances One such mechanism is the conjugation of glycuronic acid derived from carbohydrate with poisons which possess a hydroxyl group (52, 53) Indeed, this mechanism is one of the means by which the body regulates its steroid hormone metabolism and protects itself from the harm which could result from an excess of the sex hormones (54) It is also possible that the carcinogenic substances of the steroid type might be disposed of in the same man

ner Another hepatic mechanism is the acetylation of such substances as *p* amino-benzoic acid (55) and sulphanilamide (56) In this type of conjugation the acetyl groups are derived from carbohydrate probably via pyruvate and acetyl phosphate The rates of glycuronate formation and of acetylation have been shown to depend directly upon the concentration of carbohydrate in the liver (56, 57)

The protein sparing action of carbohydrate has already been mentioned This action occurs partly in the liver, for it is this organ which is primarily responsible for the deamination of amino acids Up to the point of deamination the fate of amino acids in metabolism has not been finally determined They may be used as building blocks from which to form proteins for the repair or growth of tissues, or they may be broken down for use as fuel Once deamination has occurred, the amino acids are divorced from protein metabolism The amino group is converted to urea and excreted while the non nitrogenous fraction is either used as a source of energy or converted to carbohydrate or fat * The rate of deamination in the liver decreases as the available carbohydrate increases An ample supply of carbohydrate thus conserves the products of protein breakdown in a form which may be used by the body to build or maintain its own protein structure To put it in an other way, a minimal intake of protein which may be adequate for the body s needs when taken together with good amounts of carbohydrate, may become in adequate when the carbohydrate intake is deficient (58)

The availability of carbohydrate to the liver also determines how much fat is broken down by this organ There is no direct index of the rate of fat metabolism in the liver, for, unlike protein metabolism fat metabolism is not accompanied by the excretion of a characteristic end product in the urine However, it happens that fatty acids are not completely metabolized by the liver and that the end products of fatty acid metabolism in this organ are the so-called 'ketone bodies" *β* hydroxybutyric and acetoacetic acids (59 60, 61) These ketone bodies must then go to the peripheral tissues for complete oxidation Ordinarily the rate of breakdown of fat and of the formation of ketone bodies is such that the latter are promptly disposed of by the peripheral tissues, so that no significant amounts ap pear in the blood or urine But when fatty acid breakdown becomes excessively rapid and the rate of ketone formation in the liver begins to exceed the rate of dis posal by the peripheral tissues, there begins to occur an accumulation of the ke tone bodies in the blood and an excretion of these substances in the urine (ketosis) Under these circumstances in an otherwise normal animal the administration of carbohydrate causes a prompt disappearance of the ketone bodies (antiketogenic action) This effect of carbohydrate occurs in the liver and is due to an inhibition of the breakdown of fatty acids Together with the protein sparing action of carbohydrate, its antiketogenic action serves to regulate the proportion of the

* Under certain circumstances the non nitrogenous fraction may also be reaminated and restored as an amino acid (chap u p 39)

different foodstuffs which are prepared by the liver for use as fuel by the peripheral tissues

In discussing the special functions of carbohydrate in the liver we have referred both to its "*glycogen content*" and to the "*availability*" of carbohydrate to this organ These terms may or may not be synonymous, for it is still not known whether sugar may be used directly by the liver cells or must first be built up to glycogen In any case, the glycogen content of the liver is a good index of the *amount of carbohydrate which is available to the hepatic cells*, and from a nutritional standpoint it is important to remember that carbohydrate is the foodstuff which leads to the highest levels of liver glycogen Fairly good glycogen stores in the liver can be obtained when protein is predominant in the diet, while a high fat *diet results in a liver which is poor in glycogen* (62, 63) The medical uses of the high carbohydrate diet or of the intravenous administration of dextrose solution are directed toward the protection of the liver by insuring rich glycogen stores (50) Protein has been used with the same ultimate purpose in mind, but it is less effective, probably in proportion to its convertibility to sugar

Carbohydrate and the heart—The previous discussion of carbohydrate as the most efficient fuel of muscular exercise, and of the muscle glycogen as an important emergency source of contractile energy, applies in even greater measure to cardiac *muscle than it does to skeletal muscle The latter can in some measure accommodate* itself to a decreased supply of carbohydrate by decreasing its work The heart cannot stop to rest A temporary reduction in the supply of sugar to the normal heart (as in induced attacks of hypoglycemia) has little apparent effect on the organ, although a definite change in the electrocardiogram may be noted (64) The apparent lack of influence of hypoglycemia on the normal heart may be due to the good glycogen stores to be found there But, in the heart which is damaged by disease and in which the initial glycogen stores are poor, hypoglycemia may precipitate stenocardial symptoms with angina and may even result in death This has been noted for diabetic (65), as well as for non diabetic, cardiac patients, and in both it has also been observed that they may do better when the blood sugar is somewhat elevated even above the normal range High carbohydrate therapy has been successfully used on this basis (66)

The indispensability of carbohydrate to the central nervous system—Of all the organs and tissues in the body, the central nervous system is most dependent upon the minute by minute supply of glucose from the blood In connection with the discussion on the fuel of muscular exercise it was stated that carbohydrate was of primary importance, while protein and fat could be used only indirectly As regards the central nervous system, it has been well established that only carbohydrate can be used (67, 68, 69) The need of nerve tissue for glucose is even more specific than the previous statement would indicate It is true, when slices of brain

tissue are studied *in vitro* regarding their ability to maintain respiration at the expense of various substrates, that a number of degradation products of glucose will serve as well or better than glucose itself (67) However, none of these intermediates have been shown to have any ameliorating effect upon the hypoglycemic symptoms caused by lowering the blood sugar level *in vivo* (70) In other words, glucose as such has a specific influence and is indispensable for the maintenance of the functional integrity of the nerve tissue When the blood sugar is lowered in a living organism, those tissues which have ample stores of glycogen may use the latter to tide them over the lean period The nervous tissue has little glycogen, and it is doubtful whether the little which is present can be mobilized for use in emergencies The glycogen content of nervous tissue remains more or less constant under most conditions, including hyperglycemia and hypoglycemia, and may be largely an integral part of the nerve structure (34) The unavailability for metabolic use of the glycogen present in the nerve cells is evidenced by the dramatically rapid development of hypoglycemic symptoms when the blood sugar is lowered

THE TRANSFORMATION OF CARBOHYDRATE INTO FAT

In the previous discussion of fat as a fuel storage material it was pointed out that, when food in excess of caloric expenditure is ingested (whether in the form of carbohydrate, protein, or fat), the equivalent of the excess calories is deposited as fat in the adipose tissues With this in mind, it is, strictly speaking, incorrect to label any of the foodstuffs as being particularly "fattening " Any one of them can be so if taken in sufficient quantities But because of its proportion in the diet, its lower cost, and its use in confections, carbohydrate is quantitatively the most important precursor of fat

The fat which arises from carbohydrate in the body is the so-called "hard" fat, composed, in the main, of the highly saturated palmitic and stearic acids (71) This is probably of more concern to stock raisers than to human nutritionists The former have long known that they could control the physical qualities of the fat in meats by varying the proportion of carbohydrate and of oils in the diet of their animals Of course, carbohydrate cannot completely substitute for fat in the diet, since it does not carry with it the essential fatty acids and the fat soluble vitamins, which cannot be manufactured by the body

THE INTERRELATION OF CARBOHYDRATE AND PROTEIN METABOLISM

Earlier writers on metabolism have talked somewhat loosely of the formation of protein from carbohydrate Strictly speaking, such a transformation does not occur, because the amino groups which characterize the building stones of proteins are derived from amino acids or proteins which are ingested as such Schoen-

heimer (25) has demonstrated that, when ammonium salts are ingested, the NH_3 may combine with carbohydrate derivatives to form amino acids But what ordinarily occurs is the exchange of the amino group of an amino acid with the keto group of a keto acid (derived from carbohydrate), a process known as "transamination" (72, 73) In this process the carbon residue of the amino acid reverts to a carbohydrate intermediate, so that there is not necessarily any quantitative increase in the amount of protein precursor resulting from the reaction What the body gains from the interchange is the ability to transform one amino acid, which it may have in excess to another, which it may need For example, by exchanging with α ketoglutarate, alanine may be transformed to glutamic acid, with pyruvic acid as the by product (Fig 3)

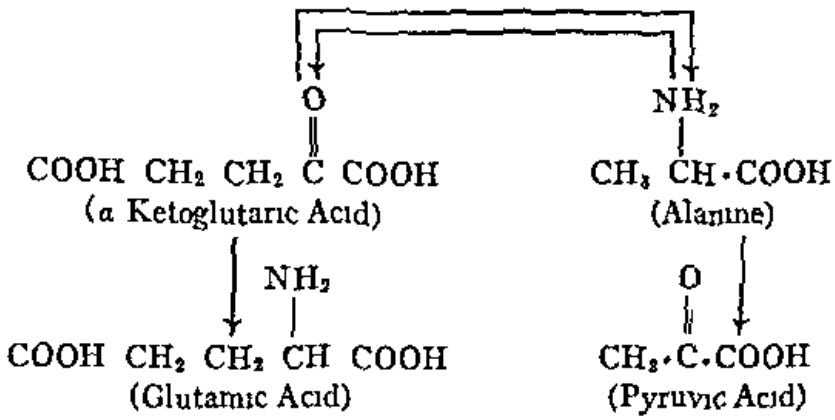

Fig 3 —Example of transamination

THE IMPORTANCE OF THE VITAMIN B COMPLEX IN CARBOHYDRATE NUTRITION

It is now known that many members of the vitamin B complex play an integral part in carbohydrate metabolism and that the requirement for this group of vitamins depends upon the amount of carbohydrate which is eaten Since this is so, why did not the knowledge of its existence arise much earlier in human experience and why did not the race suffer from the lack of such knowledge? The answer to these questions lies in the fact that it is only in comparatively recent times that the natural union between the vitamin B complex and carbohydrate, which exists in whole grain and plants has been broken by the industrial processing of foods Before this occurred, the supply of the B vitamins was automatically adjusted to the amount of carbohydrate eaten, so that the occurrence of vitamin B deficiency, with its consequent disturbance in nutrition, is a comparatively recent development in the Western world In the Orient the earlier large scale introduction of polished rice led to the first known instances of vitamin B deficiency (beriberi) and, indeed to the first recognition of the existence of this group of vitamins (74)

The vitamins, as the name signifies, were first regarded as mysterious elements,

essential for life As the different vitamins were successfully recognized and extracted in concentrated form from their natural sources, experimentation with these products led to the recognition of definite clinical syndromes resulting from their lack and cured by their administration More recently the actual chemical identity of many of the vitamins has been established, and a number of them have been synthesized Coincidentally with the latter events, the development of tissue-enzyme chemistry has revealed a great deal about the chemical steps through which the foodstuffs are broken down and used for energy It is now known that each of the chemical steps is accomplished by the activity of one or more enzymes (protein catalysts) and that each of the enzymes requires one or more cofactors for its optimal activity In some instances the cofactor is a simple mineral substance, like iron or magnesium or phosphorus, in other cases the cofactor is a more complex organic substance, known as a "coenzyme " Thus far, those vitamins whose functions are known have been found to be coenzymes or to give rise to coenzymes in the body (75)

Figure 4 outlines the known steps in the breakdown of carbohydrate and indicates the points at which the various components of the vitamin B complex play an essential role The role of various minerals in carbohydrate metabolism is similarly indicated It may be seen that definite knowledge is available regarding only three B factors, namely thiamine nicotinic acid, and riboflavin It is to be expected that similar functions will eventually be found for the other factors in the B complex

Since the breakdown of carbohydrate is essentially similar in all tissues and organs, it follows that a vitamin B deficiency will impair carbohydrate metabolism in every structure of the body The clinical syndromes which have been described are, therefore merely the most obvious manifestations occurring in those tissues and organs that suffer most acutely and that are most easily accessible to examination Consideration of Figure 4 also shows the fallacy of regarding any single factor of the B complex as more important than another, for the normal chain of events can be broken by a lack of any one of them For this reason and until we have isolated and know the precise function and optimal proportion of each component part of the B complex, a natural source containing all the factors remains the best protective dietary supplement with which to avoid the evils of modern food refinement

THE UTILIZATION OF SIMPLE SUGARS OTHER THAN GLUCOSE

In the previous section on the distribution of carbohydrate in the body it was pointed out that all the hexoses absorbed from the gastro-intestinal tract are converted into either glucose or glycogen This conversion, which takes place largely in the liver, is ordinarily so efficient that there is little need to consider any other fate which sugars like fructose and galactose may undergo However, under special

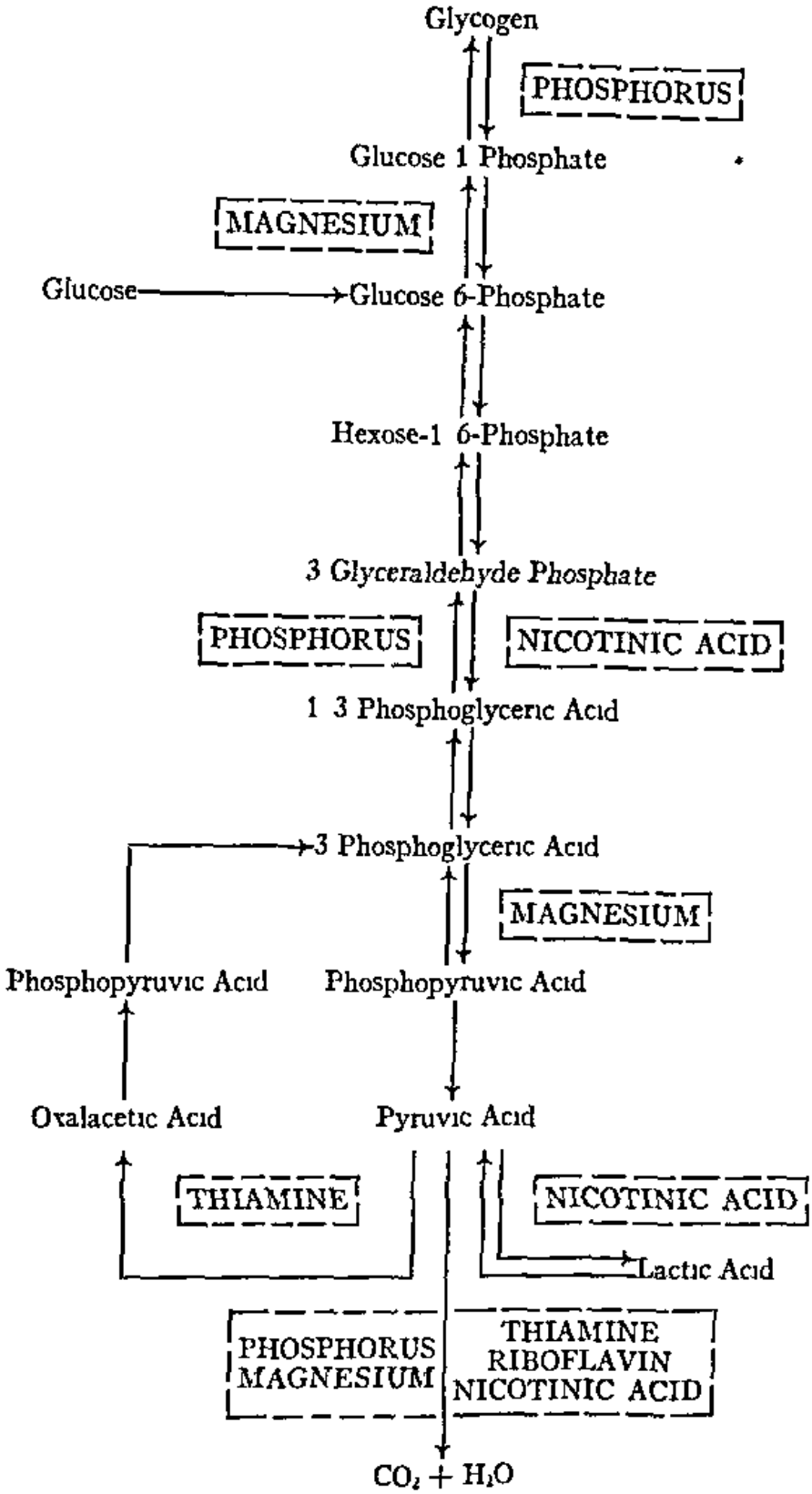

FIG 4 —Points of action of vitamins and minerals in carbohydrate metabolism The substances required for a particular reaction are necessary in both directions of the reaction

circumstances when the function of the liver is impaired or when these sugars enter the blood in overwhelming quantities there occur interesting anomalies of carbohydrate nutrition which deserve some brief mention Lactose is also of interest because of its formation in large quantities by the lactating breast of the female at which time it may appear in the blood and the urine The pentoses are sometimes involved in a hereditary anomaly of metabolism

a) Fructose —While the conversion of fructose to glucose occurs largely in the liver, there is some evidence that it may take place to a smaller extent in the intestinal mucosa and the kidney (28 29 76) Recent work indicates that there are probably two chemical pathways from fructose to glucose in the liver The fructose may be phosphorylated to fructose 6 phosphate which is converted to glucose 6 phosphate and then split by the liver phosphatase to yield glucose (77) The phosphorylated fructose also appears to be more readily degraded to lactic acid than is glucose 6-phosphate Hence when fructose appears in excess in the blood it is accompanied by a rise in lactic acid (78) Some of the latter may be converted to glucose or glycogen by the liver

When any of the foregoing hepatic mechanisms are impaired either by liver disease or by a hereditary anomaly known as essential fructosuria there is difficulty in disposing of the fructose taken in through the gastro-intestinal tract and it accumulates as such in the blood (79) Since it is a substance which is not held back by the kidney as efficiently as is glucose it appears in the urine in abnormal quantities Fructose is a reducing sugar which is not distinguished from glucose by the routine chemical tests From the medical standpoint it is therefore important not to confuse fructosuria with diabetes mellitus

b) Lactose and galactose —Lactose is split into glucose and galactose in the process of digestion It may therefore be considered together with the galactose which is ingested as such However the presence of lactose in milk and milk products renders it much more important than galactose from the nutritional standpoint Lactose also has the special virtue of altering the intestinal flora in such a manner as to produce a more acid environment which favors the more complete absorption of ingested calcium (80)

There is some recent evidence that suggests that galactose is converted to glucose in the liver by phosphorylating steps similar to those described for fructose (81) Little beyond this is known For example the lactating breast manufactures lactose and presumably has galactose available for the purpose (82) but it is not known whether all the galactose is made in the breast or whether some of it originates in the liver and is transported to the breast Both lactose and galactose may be found in the blood and urine of lactating females so that the mere presence of these abnormal constituents does not give any indication as to their site of origin As with fructose it is of importance medically to distinguish between galactosuria lactosuria and glucosuria

In the previous discussion of the special functions of carbohydrate in the liver mention was made of its protective and antiketogenic action Liver glycogen that is formed as a result of the intake of galactose or of lactose may perhaps be more beneficial to the organism than glycogen that originates from other materials This is because, for some unknown reason, the "galactose glycogen" is more stable It has been shown that, when galactose is administered to animals together with a ketogenic agent, the ketosis which follows is less than when glucose or fructose are similarly administered (15)

c) Pentoses —In contrast to the hexoses, which are important energy materials the five carbon atom sugars are much more important as part of the machinery of the body Pentoses are incorporated in at least one vitamin (riboflavin), several tissue coenzymes (diphosphopyridine nucleotide, triphosphopyridine nucleotide, and alloxazine adenine dinucleotide), and all the nucleoproteins However, when pentoses as such are ingested, they are not utilized but are eliminated, more or less quantitatively, in the urine and feces It is possible that the pentoses which are eaten in combined form as part of natural food constituents (riboflavin and the nucleotides, for example) do contribute to the pentose content of the tissues It is known that the body is able to synthesize pentoses for itself from glucose by way of glycuronic acid (83) The hereditary anomaly known as "essential pentosuria" is as yet unexplained

SUMMARY

We have seen that carbohydrate is not only the primary fuel of the body but is also involved in important portions of its functional machinery The carbohydrate stores, though relatively small as compared to fat, play a protective role in some of the most vital organs They may be of the utmost importance when a rapid source of energy is required, to enable the organism as a whole to cope with an emergency in its environment Despite all this, however, the evolutionary processes have resulted in so flexible a metabolic system that the higher mammals and man can get along very nicely when little or no carbohydrate is available Under these circumstances the body makes its own carbohydrate fuel from non carbohydrate materials But this is a wasteful process, because some energy must be used for the conversions, and there is more wear and tear of the metabolic machinery

If, with the foregoing considerations in mind, we could divorce ourselves from previous dietary experience and were to attempt to construct an ideal adult diet, we would choose the following

1 Protein sufficient in quantity and quality to repair the protein machinery from day to day, and a little extra, to be on the safe side In the same category we would place a sufficiency of all the vitamins and minerals

2 Enough fat to carry the essential fatty acids and fat soluble vitamins and to make it unnecessary to eat too large a bulk of other food

3 Carbohydrate sufficient to supply all the rest of the calories necessary to maintain weight

The diet which has been outlined is a fair approximation of that which the human race has actually adopted on the basis of experience, in those fortunate parts of the world where food resources are rich and the choice is not limited (84)

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CHAPTER II

THE ENZYMATIC MACHINERY OF CARBOHYDRATE METABOLISM

IN THE process of digestion or in the liver after absorption, carbohydrates are largely converted to glucose Hepatic gluconeogenesis leads to the same end product The further course of carbohydrate metabolism is therefore chiefly concerned with the chemical transformations undergone by glucose These include the synthesis of glycogen and the formation of fat But more basic than either of these is the breakdown of the sugar to carbon dioxide (CO_2) and water (H_2O), with the liberation of the energy that supports the various functions of living cells

Lavoisier's analogy of the burning candle introduced the concept of oxidation in the living organism and the use of the term "combustion" to describe the ultimate breakdown of foodstuffs in the body The analogy was apt and useful at the time The living organism, like the burning candle, required oxygen and produced CO_2 and H_2O What could be more natural than the conclusion that the lungs served as a furnace, where the inspired oxygen united with carbon and hydrogen from the blood to produce heat, energy and the appropriate end products (1)? During the first half of the nineteenth century the discovery that the blood contained O_2 and CO_2 resulted in a shift in the location of the theoretical furnace from the lungs to the blood (2) However, the development of histological and biochemical techniques soon led to the realization that the individual tissue cells were the functional units of metabolism, while the blood served mainly as a medium of transport (3) This, in turn gave birth to the vague and somewhat vitalistic conception of the ability of the body tissues to ' oxidize" food materials and to derive heat and energy therefrom At that time, the word "oxidation" was not used in the strict chemical sense of today As then used, it meant the simple addition *of oxygen to molecules or carbon fragments of the original foodstuffs within the tissue cells, and the liberation of energy by complete oxidation of the foodstuffs to* CO_2 and H_2O This conception, with little modification, has been carried forward in some writings to the present day

The work of Pasteur on yeast fermentation initiated a series of scientific developments, which at first were apparently unrelated to the above but which eventually merged completely The epoch making discovery by Buchner (4) that *a cell free extract of yeast could substitute for the living cell in the process of fer*mentation showed that what had been considered to be a process inseparable from

ife is, after all, only a special kind of chemical reaction—a reaction that is catalyzed by a complex organic substance (enzyme) in the cell This paved the way for a rational and materialistic explanation of cell processes Other enzymes were discovered and isolated Evidence mounted that the chemical machinery of the living cell consists of a series of organic catalysts which operate on complex molecules, step by step, to produce simpler and more labile products It was realized that the enzymes made possible such chemical reactions in the cell as would otherwise require high temperatures or strong reagents incompatible with life The step by step catabolism controlled by the multiple enzymes also offered a reasonable basis for the regulated release of energy in small units, a process which was much more reasonable, from the point of view of the use of such energy, than the explosive type of reaction, implied in the idea of "combustion "

By the early years of this century biochemists and physiologists using biochemical methods had collected a great deal of data concerning the kinds and amounts of intermediate metabolites present in the different tissues of the body under a variety of conditions These data guided the enzyme chemists in the isolation and study of the enzyme systems which were responsible for the various products The last ten to fifteen years have witnessed a tremendous and constantly accelerating growth in the application of enzyme chemistry to metabolic problems It has become evident that, in the process called "oxidation' in the tissues, molecular oxygen does not interact directly with the foodstuffs (5, 6) and that CO_2 largely arises by a splitting-off of carboxyl groups from lower metabolic intermediates (7) It is with these and other fundamental enzyme reactions that the present chapter will deal

NATURE OF CELL ENZYMES

The enzymes in the living cell resemble the known inorganic catalysts in that they are more or less specific for a particular chemical reaction or type of reaction, also, in that they are not measurably consumed by the reaction which they accelerate All the tissue enzymes which have thus far been isolated and sufficiently purified that their essential natures are known have turned out to be proteins (8, 9) As more and more of the enzymes have been recognized and studied, it has become less possible to distinguish between purely structural proteins constituting, as it were, the skeleton of the cell (10), and the enzyme proteins, representing the active organs of the cell In fact, a tabulation of the number of enzymes present in skeletal muscle and a calculation of the proportion of the total cell protein which enzymes must represent leaves little or no room for the presence of any purely structural proteins (Table 4) (9 11)

Studies of the optimal conditions for the activity of various enzyme proteins have uncovered a number of other normal constituents of the living cell which must be present if a particular enzyme is to exert its fullest effect In some in

stances these accessory substances are simple ions, like phosphate or magnesium, and are referred to as "cofactors" of the enzyme When the accessory element is a complex organic but non protein substance, it is known as a "coenzyme" (12) A protein enzyme (or the activating protein) together with its particular coenzyme and/or other cofactors is known as an "enzyme system"

THE ENZYME SYSTEMS INVOLVED IN CARBOHYDRATE METABOLISM

The following is a list of the various types of enzymatic reactions which are known to be involved in the breakdown and synthesis of carbohydrates in mammalian tissue The enumeration is followed by a brief description of the nature of

TABLE 4

PROPORTION OF THE MUSCLE PROTEIN ACCOUNTED FOR BY A FEW OF THE MANY KNOWN ENZYME SYSTEMS*

Catalytic System	Percentage of Total Protein	Reference
Adenosinetriphosphatase (myosin)	50–60	Engelhardt (11)
Zymohexase (myogen)	2	Herbert (102)
Lactic dehydrogenase	0 4	Straub (15)
Cytochrome C	0 09–0 3	Stotz (92)
Myoglobin	0 5 –1 0	Millikan (103)

* There are at present forty additional known enzyme systems in the muscle cell (9) Their relative concentrations are unknown It is evident however that practically all of the cell proteins are constituents of active catalytic systems

each reaction and an important example of each type, including mention of the coenzymes and cofactors involved

1 Oxidation (oxidoreduction)
2 Decarboxylation (oxidative and non oxidative)
3 Carbon dioxide assimilation (addition of CO_2)
4 Phosphorylation and phosphorolysis
5 Intramolecular phosphate transfer
6 Deamination
7 Amination
8 Transamination
9 Hydrolysis

1 *Oxidation* —The term "oxidation" may be applied to a reaction when there is (*a*) the addition of oxygen atoms to a substance, (*b*) the removal of hydrogen atoms from a substance, or (*c*) the removal of electrons from a substance (13, 14) The transformation of lactic to pyruvic acid is such a reaction and may be indicated as follows

1 mol lactic acid − 2 hydrogen atoms = 1 mol pyruvic acid

The hydrogen is not given off in gaseous form but rather in the form of hydrogen ions and electrons This means that for each hydrogen ion one electron is also released The correct chemical notation for this reaction is therefore

$$CH_3 \cdot \overset{a}{C}HOH \cdot COOH - 2H^+ - 2_e = CH_3 \cdot CO \cdot COOH$$

Since this particular oxidation consists of the removal of hydrogen atoms, it is often referred to as a "dehydrogenation"

Lactic acid, dissolved in H_2O and with free access to oxygen at 37°5 C, will be oxidized to pyruvic acid at such a slow rate as to be hardly measurable But when a specific protein derived from animal or plant cells is added to the solution, significant amounts of pyruvic acid appear in a matter of minutes (15) This influence of the activating protein or enzyme may be regarded as one which loosens the bonds joining the two hydrogen atoms to the second, or αC, atom of the lactic acid molecule More accurately stated, the activating protein changes the form of the electron energy, uniting the hydrogen and carbon in such a way as to increase the tendency of the hydrogen atoms to fly off (16) Thus any suitable chemical substance which can bind the hydrogen atoms (hydrogen acceptor) will remove the "loosened hydrogen from the orbit of the lactic acid, leaving pyruvic acid (Fig 5) (17, 18, 19)

The hydrogen acceptor necessary for the above reaction is diphosphopyridine nucleotide (DPN) (Fig 6) (15) This, then, is the coenzyme which, together with the protein, makes up the lactic acid oxidase (or dehydrogenase) system Despite this nomenclature, however, the system is reversible and will actually reduce pyruvic acid to lactic acid under the proper conditions (17) The direction of the reaction depends largely on whether the DPN is present in its oxidized or reduced form (as DPN or as H_2DPN) which, in turn depends upon whether other systems which can remove the hydrogen from DPN are present (20, 21) For example, the activity of the lactic acid oxidase system in the living animal is most frequently observed during relative or absolute anoxia in skeletal muscle when the H_2DPN cannot readily be reoxidized and hence serves to convert pyruvic acid to lactic acid In chemical notation the reaction may therefore be represented somewhat more completely, as follows

$$CH_3 \cdot CHOH \cdot COOH + DPN \underset{\text{protein}}{\overset{\text{activating}}{\rightleftharpoons}} CH_3 \cdot CO \cdot COOH + H_2DPN$$

While the activating protein of the lactic acid oxidase system is completely specific for the one substrate, lactic acid, and is just as specific for the particular transformation of lactic acid which we have described, the coenzyme is less discriminating It also serves as a hydrogen acceptor for other reactions (see Table 5) Each of these reactions is catalyzed by a separate activating protein in combination with DPN Some biological oxidations are carried on by systems consisting of proteins and TPN (see legend to Fig 6) These two groups constitute the class of pyridinoprotein enzymes (22, 23) Another group of oxidation systems are known as the "yellow enzymes —proteins combined with alloxazine derivatives (Fig 7), which are yellow in aqueous solution (24, 25)

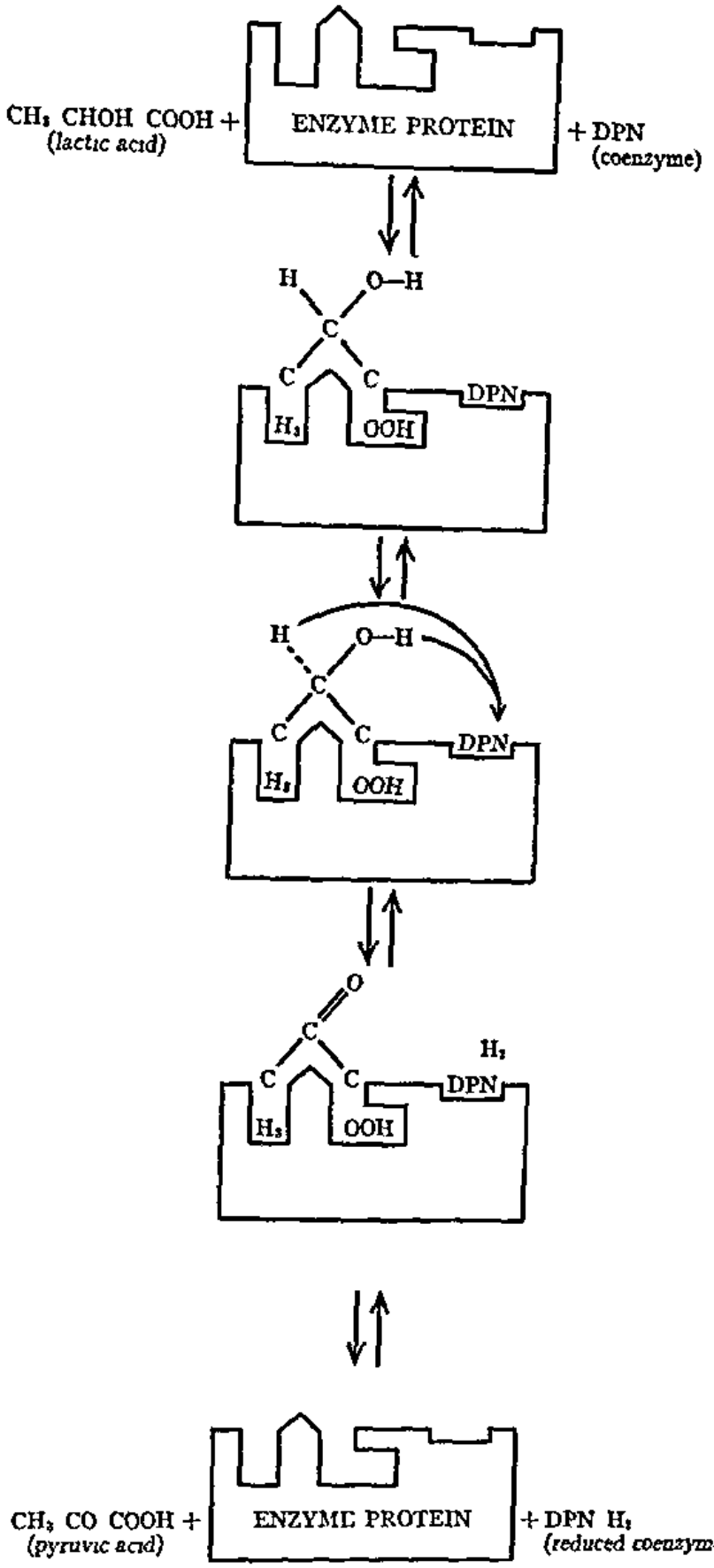


FIG 5 —A schematic representat on of the configurat on of an enzyme prote n (magmary) showing the manner in wh ch it is thought to anchor the substrate and the coenzyme and to facil tate the interaction between the free groups of both

The various oxidation systems that have been listed are responsible for the removal of hydrogen from all substrates and intermediate substances whose metabolic fate is known The hydrogen removed from the original owner, while under the influence of a specific protein, is simply transferred to the coenzyme of the system, be it DPN, TPN, or an alloxazine It will be noted that no mention has been made of the appearance of oxygen upon the scene As a matter of fact, the

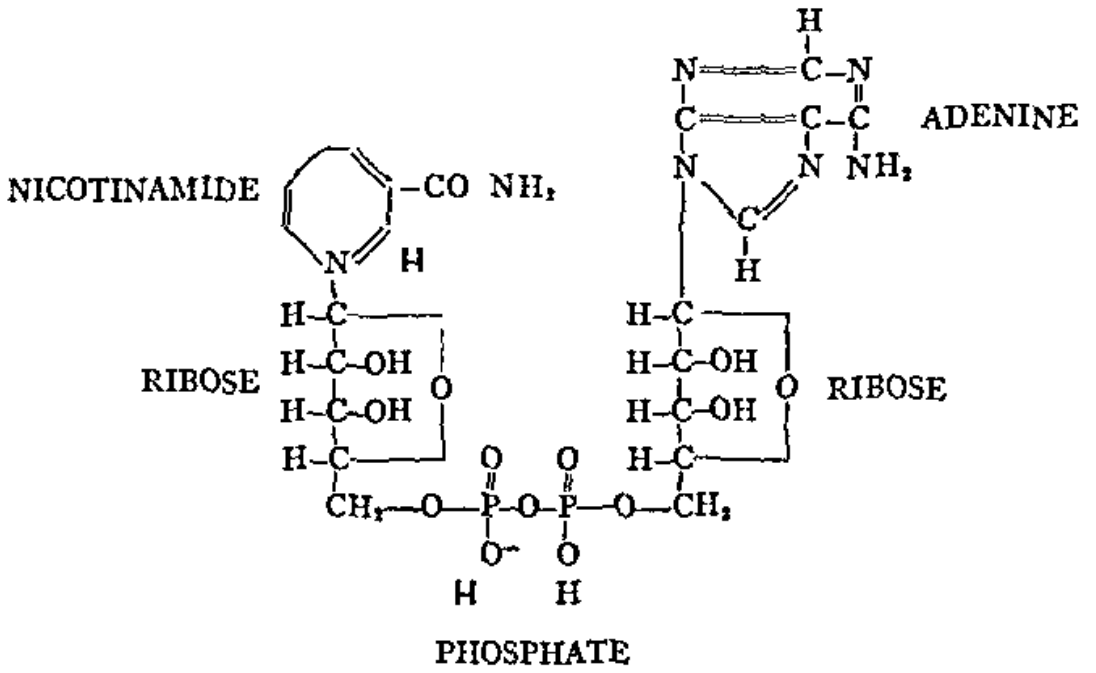


FIG 6.—Diphosphopyridine nucleotide (DPN) H = hydrogen atoms from substrate (Triphosphopyridine nucleotide (TPN) differs from DPN in possessing an additional phosphate group between the ribose units of the molecule)

TABLE 5

OXIDOREDUCTION REACTIONS AND THE COENZYMES OPERATIVE IN THEM

Reaction	Coenzyme	Reference
Lactate⇌Pyruvate	DPN	Straub (25)
Alcohol⇌Aldehyde	DPN	Lutwak Mann (26)
β hydroxybutyrate⇌Acetoacetate	DPN	Green (27)
Glucose⇌Gluconic acid	DPN	Harrison (28) Das (29)
Malate⇌Oxalacetate	DPN	Green (30)
Xanthine⇌Uric acid	Flavin	Haas (38)
Aldehydes⇌Acids	Flavin	Ball (39)
Fumarate→Succinate	Flavin	Booth (40) Gordon (41)
	Flavin	Fischer (42 43)

hydrogen seized by the coenzyme is passed on through a series of other systems in the manner of a bucket brigade before it finally arrives at the point where it may combine with oxygen to form H_2O This will be discussed in detail later (p 41)

2 *Decarboxylation* —Carbon dioxide is one of the end products of the complete breakdown of foodstuffs It is not formed as was formerly thought by the direct oxidation of the carbon by molecular oxygen but arises from the splitting off of carboxyl groups (–COOH) from intermediate organic acids which arise in the course of catabolism (7) The exact mechanism of decarboxylations is as yet obscure but we can distinguish two types the oxidative and the non oxidative In the first of these the CO_2 is split off a molecule while at the same time hydrogen

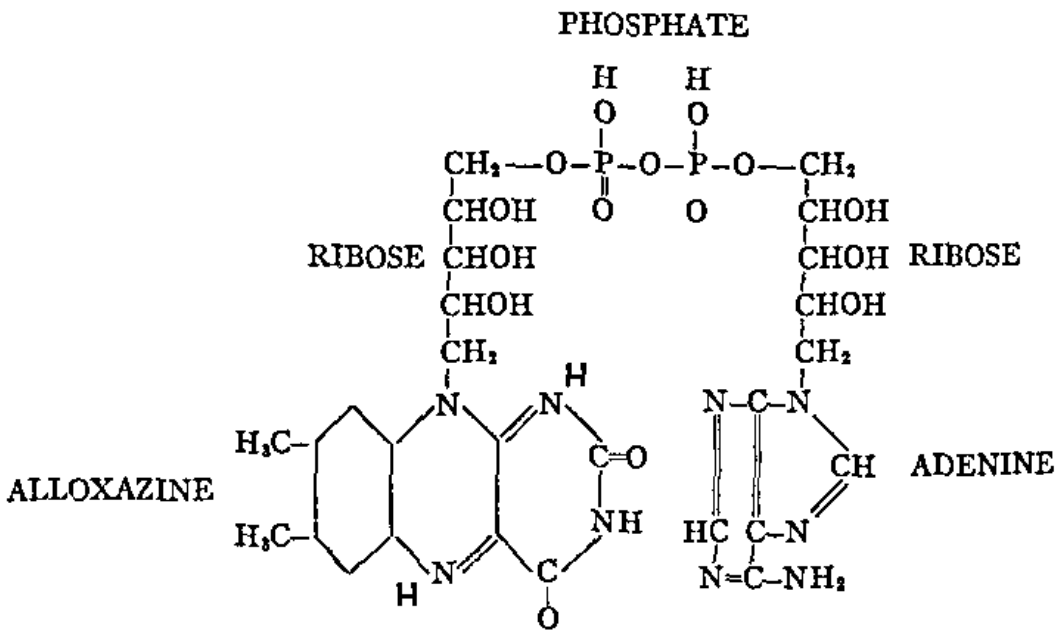


FIG 7 —Alloxazine adenine dinucleotide (flavin) H = hydrogen atoms from substrate

atoms are removed from another group in the same substance For example pyruvic acid CH_3 CO COOH containing three carbon atoms is oxidized to acetic acid CH_3 COOH which contains only two carbon atoms the third having been split off as CO_2 (44 45) In chemical notation this double process of oxidation plus decarboxylation can be presented as follows

$$CH_3\,\underset{\text{Pyruvic}}{C(=O)}\,COOH + H_2O \rightarrow CH_3\,C(OH)_2\,COOH - (2H^+ + 2) \rightarrow CH_3\,\underset{\text{Acetic}}{C(=O)OH} + CO_2$$

In the second type of decarboxylation there is no concurrent oxidation Again

using pyruvic acid as an example this type of decarboxylation proceeds as follows (46, 47)

$$\underset{\text{Pyruvic}}{CH_3\ CO\ COOH} \rightarrow \underset{\text{Acetaldehyde}}{CH_3\ CHO} + CO_2$$

Just as in the oxidations the various decarboxylations are catalyzed by specific activating proteins and the process is aided by coenzymes and cofactors The co enzyme needed for the decarboxylation of pyruvic acid is diphosphothiamine (also called "cocarboxylase) (Fig 8) Magnesium ion is also an essential compo nent as a cofactor in the foregoing systems (46 48 49)

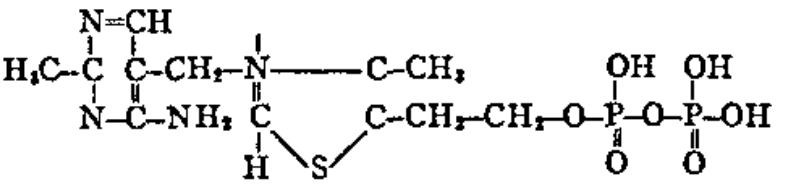


Fig 8 —D phosphothiamine (cocarboxylase)

Although the splitting off of CO_2 is not so well understood a process as is oxida tion a number of substances are known to undergo this process (Table 6) It seems quite definite that in all cases the production of metabolic CO_2 proceeds in the fashion detailed for pyruvic acid

TABLE 6

DECARBOXYLATIONS IN INTERMEDIARY CARBOHYDRATE METABOLISM

Reaction	Reference
Pyruvate → Acetate + CO_2	Lipmann (44)
Pyruvate → Aldehyde + CO	Green (48)
Pyruvate → Acetylmethylcarbinol + CO_2	Green (50)
Oxalacetate → Pyruvate + CO_2	Werkman and Wood (51)
Isocitrate → α ketoglutarate + CO_2	Krebs (7)
α ketoglutarate → Succinate + CO_2	Ochoa (52)

3 *Carbon dioxide assimilation* —It has been known for some time that CO_2 produced by the dissimilation of foodstuffs may combine with hemoglobin (car bamino compound) (53) or may be used for the production of urea (54) It was sup- posed that by these and other means all the CO_2 produced by the mammalian organism was eventually excreted by the lungs and the kidneys Only plants or certain autotrophic bacteria were thought to possess the ability to incorporate CO_2 into usable cell products (51) In 1936 this ability was first observed in bac teria (55 56), later it was confirmed for mammalian tissue (especially liver) (57, 58) that certain *in vitro* reactions undergone by compounds containing three car bon atoms (the trioses) could be speeded up if CO_2 were present in the medium It was shown that this was not a consequence of the mere presence of CO_2 but that the CO_2 took part in the reactions and was incorporated into other substances (51, 58)

Again pyruvic acid will serve as a good example *In the presence of the specific* proteins, diphosphothiamine, inorganic phosphate, and magnesium ion, pyruvic acid (a three carbon atom compound) and CO_2 will form oxalacetic acid (a four carbon atom compound)

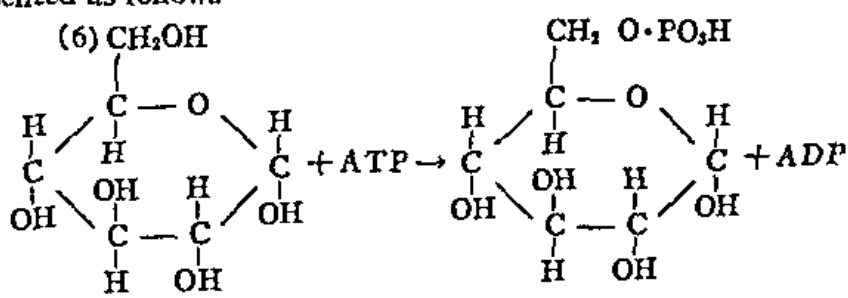
$$\underset{\text{Pyruvic}}{CH_3\ CO\ COOH} + CO_2 \rightleftharpoons \underset{\text{Oxalacetic}}{COOH\ CH_2\ CO\ COOH}$$

This is probably the first step in the series by which pyruvic acid (or lactic acid) is reconverted to sugar and glycogen (59, 60, 61)

The use of CO_2 for synthetic purposes by the mammalian cell is only now being studied in detail But it has already taken on tremendous significance, since it completely reverses the hitherto firmly accepted view that CO_2 is *merely* a waste product of animal metabolism (7, 51) It particularly affects our outlook on in direct calorimetry (p 96)

4a Phosphorylation —Early in the development of our knowledge of the enzymatic breakdown of carbohydrates it was shown that the presence of phosphate was necessary for the fermentation of glucose by yeast extracts (62) and for the breakdown of sugar that takes place in active muscle extracts (63) It was later demonstrated that the phosphate is used for the formation of various intermediaries of carbohydrate breakdown which were shown to contain phosphate in their molecules (63 64) Among such metabolites are the glucose and fructose monophosphates fructose diphosphate, glyceraldehyde phosphate, etc (cf p 50) The role of these phosphorylated intermediate substances in facilitating certain reactions and in the transfer of energy from one chemical reaction to another has only recently been elucidated We shall discuss these aspects in detail in the section dealing with the utilization of metabolic energy (chap IV, p 60) For the present it will suffice to present the mechanics of phosphorylation by suitable examples

The first step in the series of reactions by which sugar enters the metabolic cycle of the cell is the addition of phosphate (P) to the sixth carbon atom of the glucose molecule (65 66) The enzyme necessary for this initial reaction in animal tissues has not yet been purified, but it apparently activates the glucose molecule in such a way that it can receive a phosphate from a suitable source. The phosphate donor in this case is adenosine triphosphate (ATP) (Fig 9), which is the coenzyme of this phosphorylation reaction In chemical notation the reaction may be represented as follows



The coenzyme ATP has two phosphate groups, which can be split off easily in the presence of the suitable enzymes (67, 68)

$$\begin{array}{ccc} ATP & \rightarrow & ADP \rightarrow AA \text{ (adenylic acid)} \\ & & + \quad + \\ & & P_i \quad P_i \text{ (inorganic phosphate)} \end{array}$$

But the amount of ATP present in the cell at any one time is very small as compared to the amount of material to be phosphorylated Hence ADP and AA must be continuously reconverted to ATP (p 60) in order that the latter can serve as a continuous phosphate donor The central position of this adenylic system for receiving and donating phosphate groups is illustrated in Figure 10, in which the direction of the arrows represents the direction of phosphate transfer

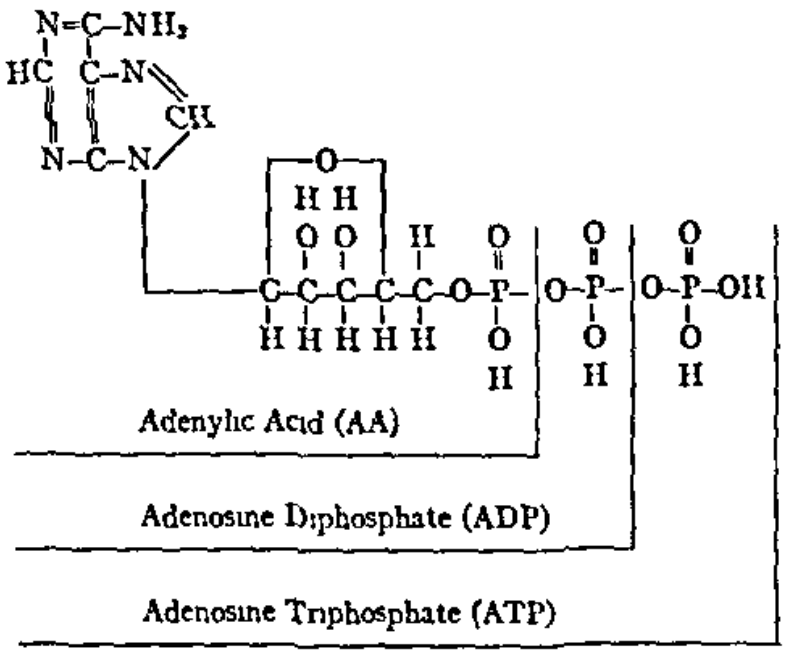


FIG 9 —The coenzyme system for phosphorylations

4*b* *Phosphorolysis* —Glycogen is a complex molecule consisting of glucose units connected to one another by glucosidic (C–O–C) linkages Two types of linkages occur, the 1 4 and the 1 6 (69 70), as illustrated in Figure 11 The glycogen complex is therefore, not a straight-chain polymer but a highly branched structure The breakdown of glycogen to hexose units is accomplished by two enzymes each of which is specific for one of the linkages The better studied and now purified system is the 1 4 enzyme, known as "glycogen phosphorylase" (71, 72) In the presence of inorganic phosphate and glycogen this enzyme catalyzes a reaction by which orthophosphoric acid (H_3PO_4) cleaves the glucosidic linkage, leaving H_2PO_4 attached to carbon atom 1 of one glucose unit and H attached to carbon atom 4 of the next glucose unit This is analogous to a hydrolytic cleavage (H OH) except that, instead of elements of H_2O, those of the orthophosphate are

added Because of this analogy the name "phosphorolysis" (compare with hydrolysis) is given to this type of reaction (104, 105, 106) The reaction is visualized in Figure 12 The 1 6 linkage is probably broken in a similar manner by the 1 6 phosphorylase (70, 72)

Phosphorolysis is reversible The direction of the reaction is determined by the relative concentrations of glucose 1 phosphate and inorganic phosphate, so that removal of inorganic phosphate favors glycogen synthesis, while addition of inorganic phosphate hastens glycogen breakdown (73, 74) There is evidence that this is one of the regulating devices of glycogenolysis in the living cell

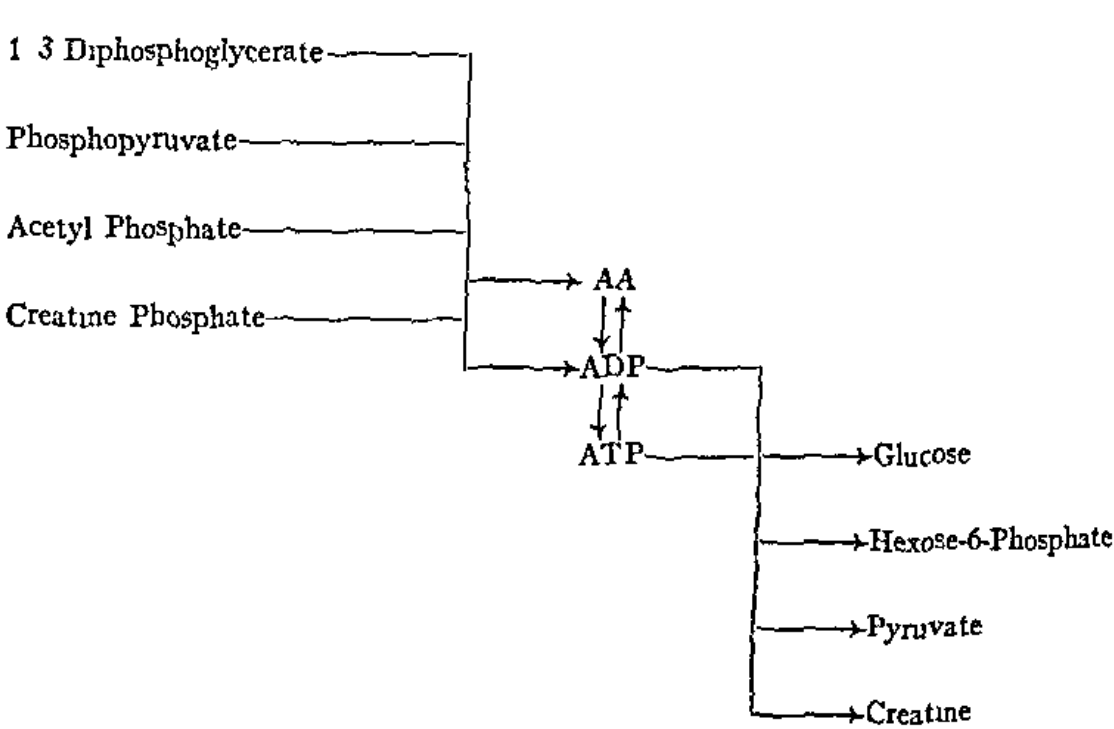
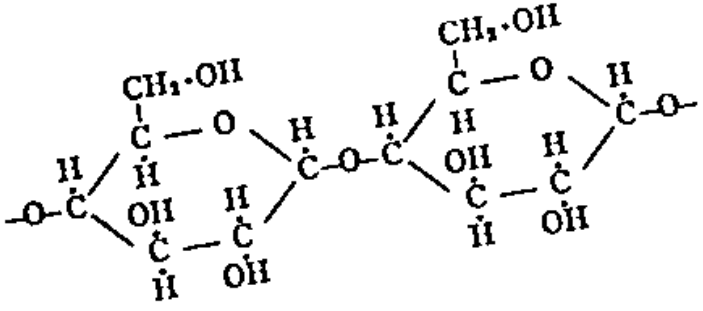


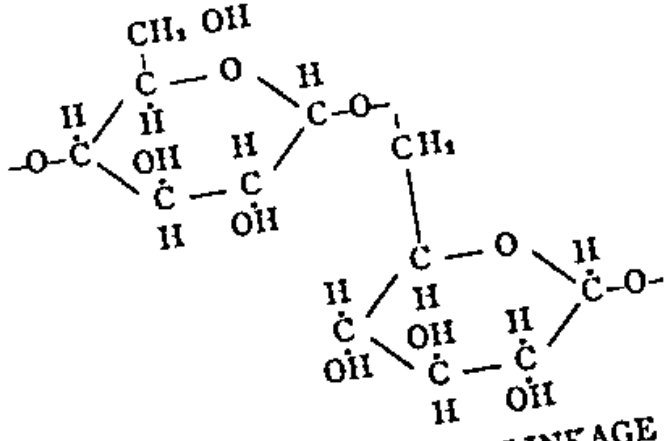
FIG 10 —Phosphate transfer by the adenylic system

5 *Intramolecular phosphate transfer* —During the degradation of glucose or glycogen certain reactions involving phosphorus occur in which a phosphate group already present in the molecule is transferred to another position in the same molecule For example, glycogen is broken down into a glucose phosphate compound in which the phosphate group is attached to carbon atom 1 of the glucose ring This is therefore known as "glucose 1 phosphate" (Glucose 1 P) An enzyme protein, called "phosphoglucomutase" (75), can then transfer the phosphate group to carbon atom 6, the resulting substance being glucose 6 phosphate (*Fig 13*) The reaction

Glucose-1 P ⇌ Glucose-6-P



THE 1 4 LINKAGE



THE 1 6 LINKAGE

FIG 11 —The structure of glycogen

is reversible, as indicated, and its necessary cofactor is the magnesium ion (75) These two phosphate glucose esters differ from each other in various chemical properties (76)

A similar intramolecular phosphate transfer occurs in the reaction

3 phosphoglyceric acid ⇌ 2 phosphoglyceric acid (77)

6 *Deamination* —The term "deamination" refers to the removal of an NH_2 (amino) group, generally from amino acids Since certain amino acids form glucose in the body and since the removal of the NH_2 group is the first step in such a transformation the mechanism of deamination is pertinent to the general discussion of carbohydrate metabolism

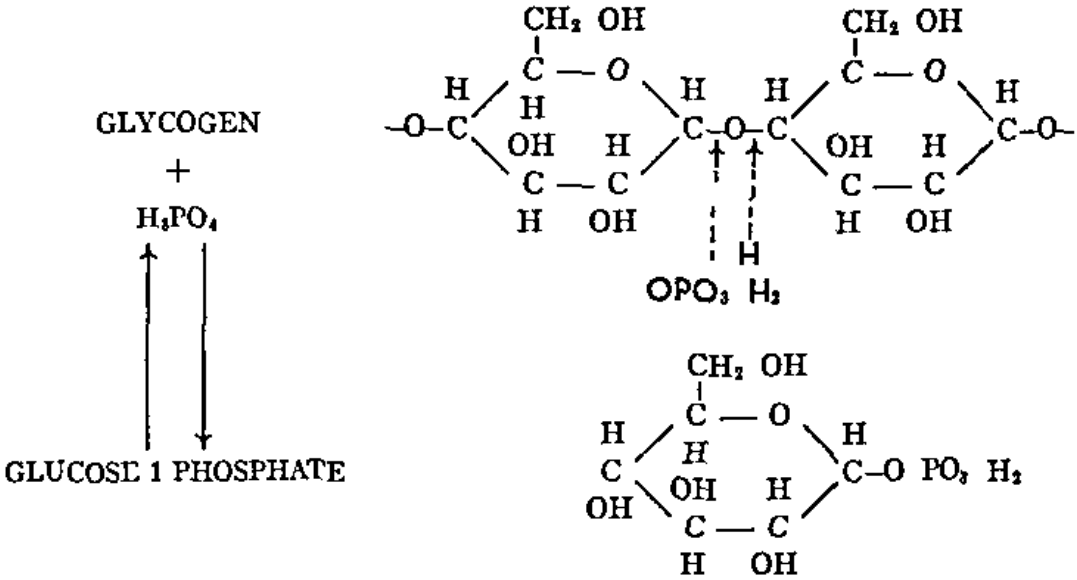


FIG 12 —Glycogen phosphorolysis

The actual loss of the NH_2 group from an amino acid is a spontaneous reaction not requiring an enzyme (36) However, the amino acid must first lose hydrogen before it can react with H_2O to lose the NH_2 group (36) Hence the whole process is called an "oxidative deamination " For example, an enzyme system known as 'amino acid oxidase,' consisting of a protein and a coenzyme of the alloxazine group removes two hydrogen atoms from the αC atom of alanine (36, 37)

$$CH_3\ \underset{\text{Amino acid}}{\overset{\alpha}{C}HNH_2\ COOH} \xrightarrow{-2H} CH_3\ \underset{\text{Imino acid}}{C\ NH\ COOH}$$

The resulting substance is known as an imino acid because of the NH or imino group Such an acid will react with H_2O as follows

$$CH_3{-}C\ \overset{\downarrow}{NH}{-}COOH + H_2O \rightarrow \underset{\text{Pyruvic acid}}{CH_3\ CO\ COOH} + \underset{\text{Ammonia}}{NH_3}$$

The final result is the formation of pyruvic acid and ammonia (37) The NH_3 produced may be excreted as such or transformed to urea The pyruvic acid is either oxidized to $CO_2 + H_2O$ or built up into glucose or glycogen

7 *Amination* —The synthesis of amino acids from the corresponding keto acids and ammonia has been suggested from model *in vitro* experiments (78), and

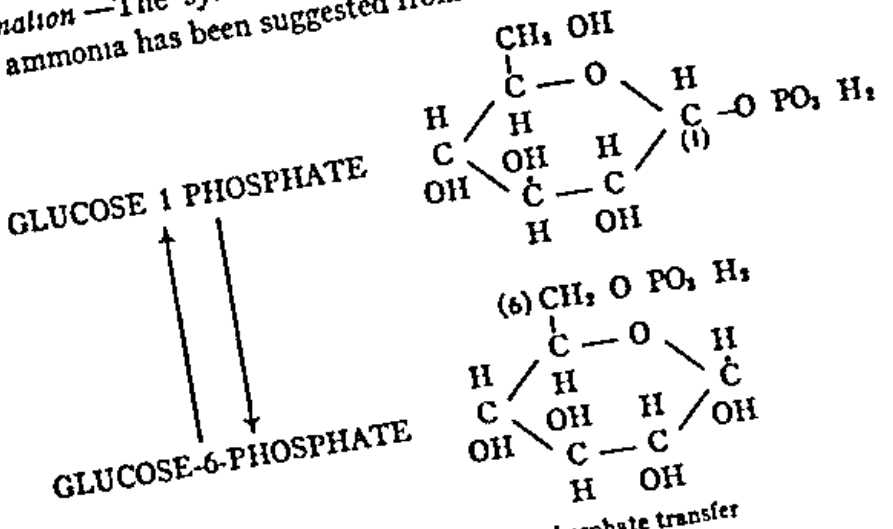
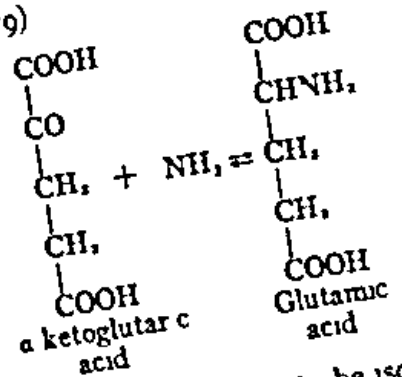


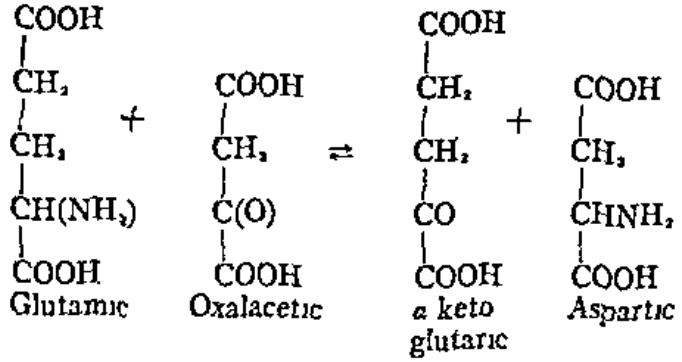
FIG 13 —Intramolecular phosphate transfer

one enzyme preparation has been shown to be able to form glutamate from α ketoglutarate plus NH_3 (79)



Although other enzymes of this kind remain to be isolated this type of reaction must be quite general for Schoenheimer has shown that following the feeding of a labeled NH_4 salt (N^{15} isotope) to experimental animals the isotopic nitrogen is found in the amino groups of all the amino acids (except lysine) of their tissue proteins (80 81) That extensive amination must occur is also shown by the fact that the corresponding keto or hydroxy acids may be substituted in the diet for the essential amino acids (82 83) Thus NH_3 like CO_2 long considered to be merely a waste product is now known to be able to re-enter the metabolic cycle and function again This must be taken into account when the urinary excretion of nitrogen is used as an index of protein catabolism (p 127)

8 *Transamination* —Another type of reaction involving amino acids and related to carbohydrate metabolism is the mutual exchange of amino and keto groups between certain α keto acids (derived from carbohydrate breakdown) and certain specific amino acids (84, 85, 86) For example



This interchange is another link between carbohydrates and protein derivatives and provides a means for the transformation of one amino acid into another It

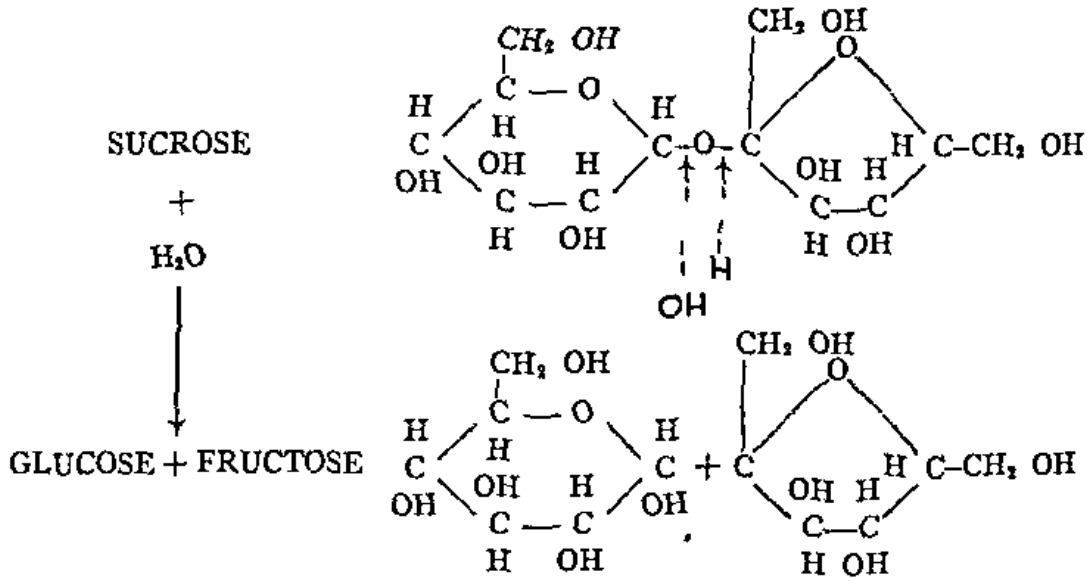


FIG 14 —Hydrolysis of sucrose

probably also represents a channel through which the amino acids contribute to the common metabolic pool formed by all the foodstuffs (see p 54)

9 *Hydrolysis* —This type of reaction is very common in the processes of digestion in the gastro-intestinal tract Water is added to a molecule in such a way that the molecule is split into two portions, one receiving the H, the other the OH group, of the H_2O (9 87) Thus sucrose, a disaccharide consisting of one molecule of glucose and one of fructose, is split into its constituent hexoses by the enzyme invertase (88) The glucosidic linkage is opened by the entry of the elements of H_2O (Fig 14)

Other examples of hydrolysis are

$$\text{Lactose} \rightleftharpoons \text{Glucose} + \text{Galactose}$$
$$\text{Maltose} \rightleftharpoons \text{Glucose} + \text{Glucose}$$

However, many reactions which formerly were thought to be examples of hydrolysis have recently been shown to be phosphorolysis, e g , glycogen breakdown (see p 35)

THE OXIDATION OF THE HYDROGEN REMOVED FROM THE SUBSTRATE

The final products of metabolism are substances which cannot be broken down further by the tissue cells These are urea, CO_2, and H, Of these, urea and CO_2 are excreted via the kidneys and lungs respectively The problem that remains is the final fate of the H, removed from the foodstuffs by the coenzymes (hydrogen acceptors) To the best of our present knowledge the sequence of events is as shown in Figure 15 The coenzymes are DPN, TPN, and flavin Although we are not in full possession of all the details, it may safely be assumed that the reduced pyridine nucleotides are relieved of their H, by flavin enzymes (20, 38, 89) A final common path for H, is reached, and all of it exists as Flavin H, for an instant The scene shifts now to a series of iron-containing proteins, the cytochromes (90, 91, 92), and the "respiratory ferment' known as "cytochrome oxidase" (93, 94, 95) The iron in these substances is in organic combination, in a group resembling the heme of hemoglobin (91) The iron can oscillate between the reduced and oxidized form

$$Fe^{++} \underset{+\epsilon}{\overset{-\epsilon}{\rightleftharpoons}} Fe^{+++}$$

by the addition or loss of an electron The H, of the foodstuffs, having arrived at the flavin stage, reacts with the oxidized cytochrome

$$\text{Flavin H}_2 + \text{CyFe}^{+++} \rightarrow \text{CyFe}^{++} + \text{Flavin} + H^+$$

The electron reduces $CyFe^{+++}$, while the H^+ remains in the medium The reduced cytochrome ($CyFe^{++}$) reacts with cytochrome oxidase

$$\text{CyFe}^{++} + \text{OxFe}^{+++} \rightarrow \text{OxFe}^{++} + \text{CyFe}^{+++}$$

This serves to restore the oxidized cytochrome and to reduce the oxidase This oxidase is unique in that it can react with molecular oxygen dissolved in the cell (93, 95)

$$\text{OxFe}^{++} + O \rightarrow \text{OxFe}^{+++} + O^-$$

The oxygen keeps the oxidase in its oxidized form and gains an electron The free H^+ available from the flavin H, then reacts with O^- to form H_2O Thus the overall change resulting from the whole series of reversible transformations is

$$2H^+ + O^- \rightarrow H_2O$$

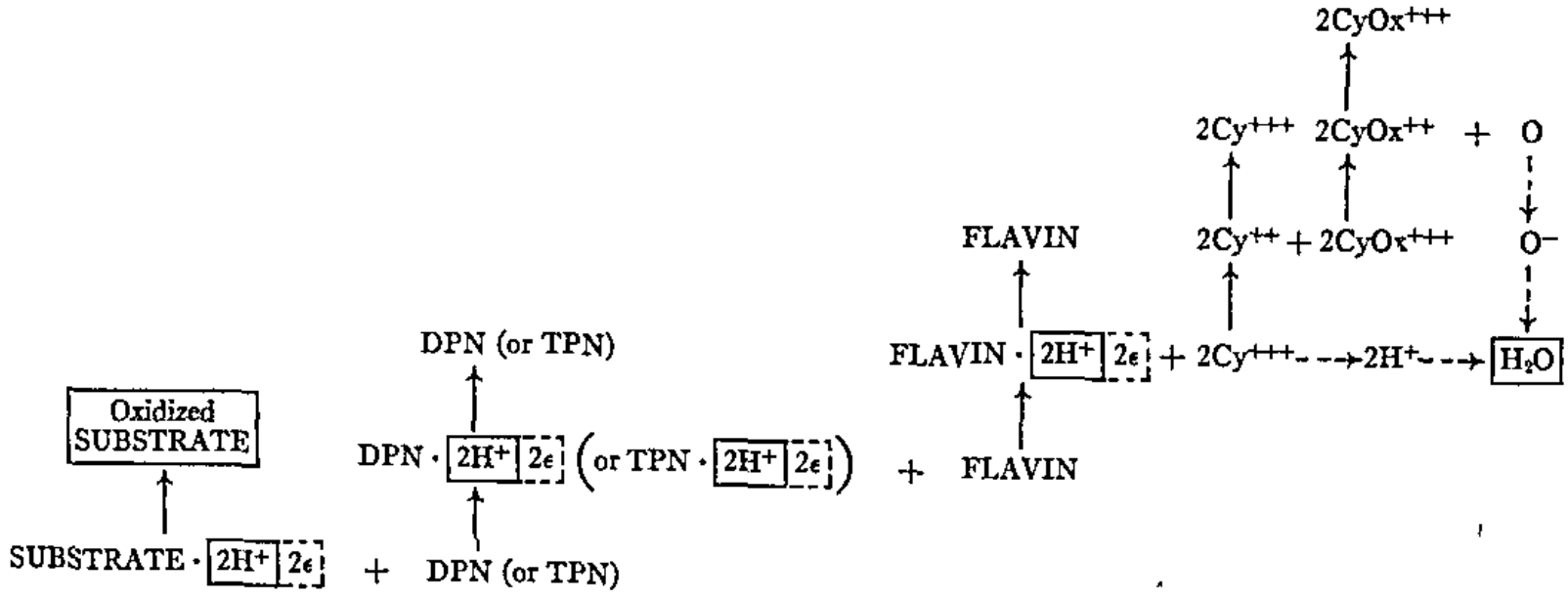


FIG 15.—Transfer of hydrogen from substrate to molecular oxygen

The series itself has been a succession of electron transfers in which every step has tended to restore the previous step to its original state

CATALYSIS BY METABOLITES

In our previous discussion of the oxidation of substrates we emphasized the role of the so called "coenzymes" as hydrogen and electron transporters They function in this way because of their ability to be reduced and then to be reoxidized so that they may serve again Many substances of a similar nature (e g , dyes like methylene blue) can function as electron mediators in certain *in vitro* biological systems under suitable conditions (96, 97, 98) These are artificially constructed pathways The cell contains certain oxidoreduction couples that can and do act like the coenzymes or the dyes (99) For example, let us again consider the oxidation of lactate to pyruvate Diphosphopyridine nucleotide serves as the coenzyme and is reduced thereby to DPN·H, The latter is reoxidized by flavin, which becomes Flavin·H, The reduced flavin may be reoxidized directly by a cytochrome, or it may be reoxidized by the couple Fumarate⇌Succinate The succinate, in turn, is reoxidized to fumarate by a specific enzyme and cytochrome C The picture of events is as follows

$$\text{Lactate}\left\{\begin{array}{ccccc} \text{DPN} & & \text{Flavin} & \text{H}_2\rightarrow & \text{Fumarate} \\ \downarrow\uparrow & & \uparrow\downarrow & & \downarrow\uparrow \\ \text{DPN} & \text{H}_2\rightarrow & \text{Flavin} & & \text{Succinate} \end{array}\right\}\text{Pyruvate}$$

It is, therefore, possible for a pair of metabolites to serve as electron and hydrogen mediators in a fashion analogous to coenzymes (6, 99) This explains why, under certain conditions, a very small amount of succinate or fumarate will stimulate oxygen consumption (100, 101) The phenomenon is referred to as the "catalysis by C_4 dicarboxylic acids" (99, 101)

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CHAPTER III

THE INTERMEDIARY STEPS IN CARBOHYDRATE METABOLISM

OUR knowledge of the intermediary steps in carbohydrate breakdown and synthesis is by no means complete However, many lines of evidence derived from studies *in vivo* and *in vitro* in animals and in plants are converging toward a generally accepted scheme (1, 2, 3) This scheme is outlined in Figures 16 and 17, which include the most thoroughly studied and in all probability, the most important pathways Others have been suggested and discarded from time to time But, of these, only certain pathways for which some evidence exists will be mentioned It should be remembered that the present scheme is subject to revision as to detail as new data appear and that it may not apply in its entirety to all organs or tissues which utilize carbohydrates (1) One or another of the enzyme systems may be missing in a particular tissue, thus modifying the intermediates or the end products The scheme, therefore, should be regarded merely as an architect s preliminary sketch, showing the general size and shape but not the final plans of the edifice to be erected

It may be seen from Figures 16 and 17 that the orderly progression of carbohydrate breakdown can be divided conveniently into two parts (1) down to the [illegible] The first [illegible] r from [illegible] phos[illegible] phorylated three carbon atom units (4) At this point the first oxidative step oc[illegible]

$$C_6H_{12}O_6 + ATP + 2(DPN) \rightarrow 2\ CH_3 \cdot CO\ COOH + 2(ATP) + 2(DPN\ H_2)$$

It should be noted that one molecule of ATP was used for phosphorylation but that two molecules were formed as a result of the oxidation of phosphoglyceralde hyde and the dephosphorylation of phosphopyruvic acid respectively This gain in ATP represents the useful energy of catabolism, as will be discussed in detail later (pp 60 ff) Meanwhile two molecules of DPN have been reduced, and in order to function again these must be reoxidized In the presence of sufficient oxygen this is probably accomplished by a flavoprotein When oxygen is lacking,

the pyruvic acid accepts the hydrogen of the DPN·H_2 and is thereby reduced to lactic acid These two alternatives may be indicated as follows

(1) $2(DPN \cdot H_2) + Flavin + Cytochrome, etc + O_2 \rightarrow 2(DPN) + 2H_2O$

(2) $2(DPN \cdot H_2) + 2CH_3 \; CO \cdot COOH \rightarrow 2(DPN) + 2CH_3 \cdot CHOH \cdot COOH$

Thus it is clear that lactic acid is not an obligatory intermediate of carbohydrate metabolism But the breakdown of hexoses to lactic acid (glycolysis) can produce useful energy and can sustain cell functions during short periods of relative or absolute anoxia

The last step above pyruvic acid, namely, phosphopyruvic to pyruvic acid, probably differs from all the others in being irreversible It is thought that when pyruvic acid is used for carbohydrate synthesis it is first transformed to phosphooxalacetic acid, which in its turn forms phosphopyruvic acid, thus reversing catabolism by avoiding the one way step (5 6)

Because of the many alternative pathways which exist below pyruvic acid, the course of its breakdown to CO_2 and H_2O is far more complex than the degradation of glucose to pyruvate Only the more important pathways are indicated in Figure 17 The orientation toward one or another path at a particular time will be determined by the equilibrium conditions, availability of catalysts, etc Despite this confusing multiplicity there has emerged from the work of Szent Györgyi (7), Krebs (2), Barron (1, 8), Wood and Werkman (9), and Evans (10) a principal scheme of pyruvate breakdown to $CO_2 + H_2O$ which is logically consistent and which helps to integrate the separate metabolisms of the three major foodstuffs

This scheme, the so-called "tricarboxylic acid cycle," envisages the formation of a six-carbon atom acid (isocitric?) by the condensation of one molecule of pyruvate with one molecule of oxalacetate The oxalacetate is itself formed from pyruvate by the addition of CO_2 (p 34) or by the deamination of aspartic acid The isocitrate formed goes through a cycle of oxidations and decarboxylations until one molecule of oxalacetate is regenerated The latter can then start the cycle off again It will be noted that the cycle begins with one molecule of oxalacetate and one of pyruvate and ends with one molecule of oxalacetate In other words, in one revolution of the cycle a molecule of pyruvate has been dissimilated, and $5(H_2)$ and $3(CO_2)$ have been produced The over all reaction can be written as follows

$$1 \text{ oxalacetate} + 1 \text{ pyruvate} + 3H_2O \rightarrow 5H_2 + 3CO_2$$
$$\downarrow$$
$$5H_2O$$

The exact mechanism of these steps is not completely understood, but there is evidence that many of the oxidative steps involved are coupled with phosphorylation, so that eventually ATP is formed (6, 11, 12) (for significance see chap IV)

FIG 16.—Intermediary steps to pyruvic acid

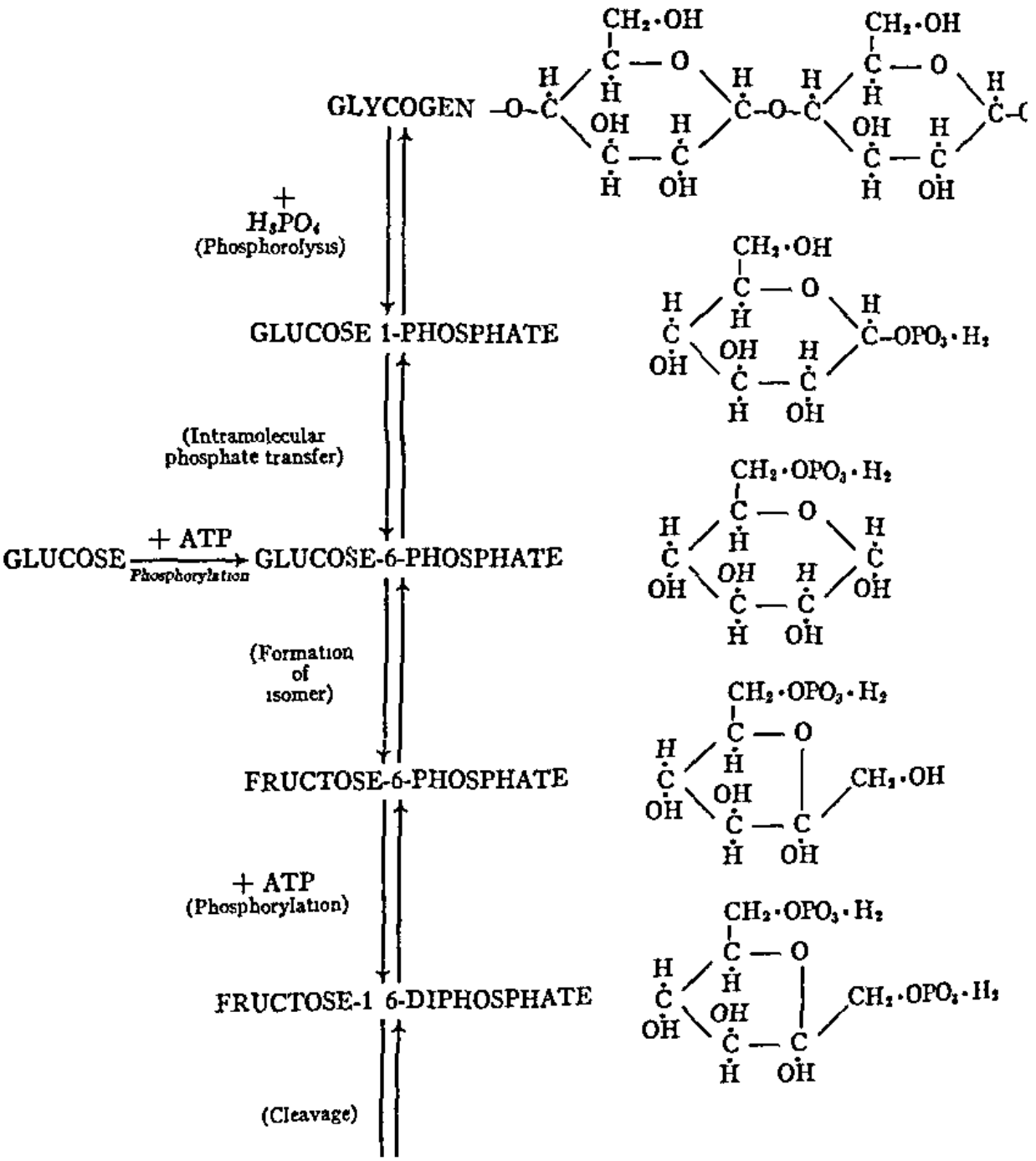
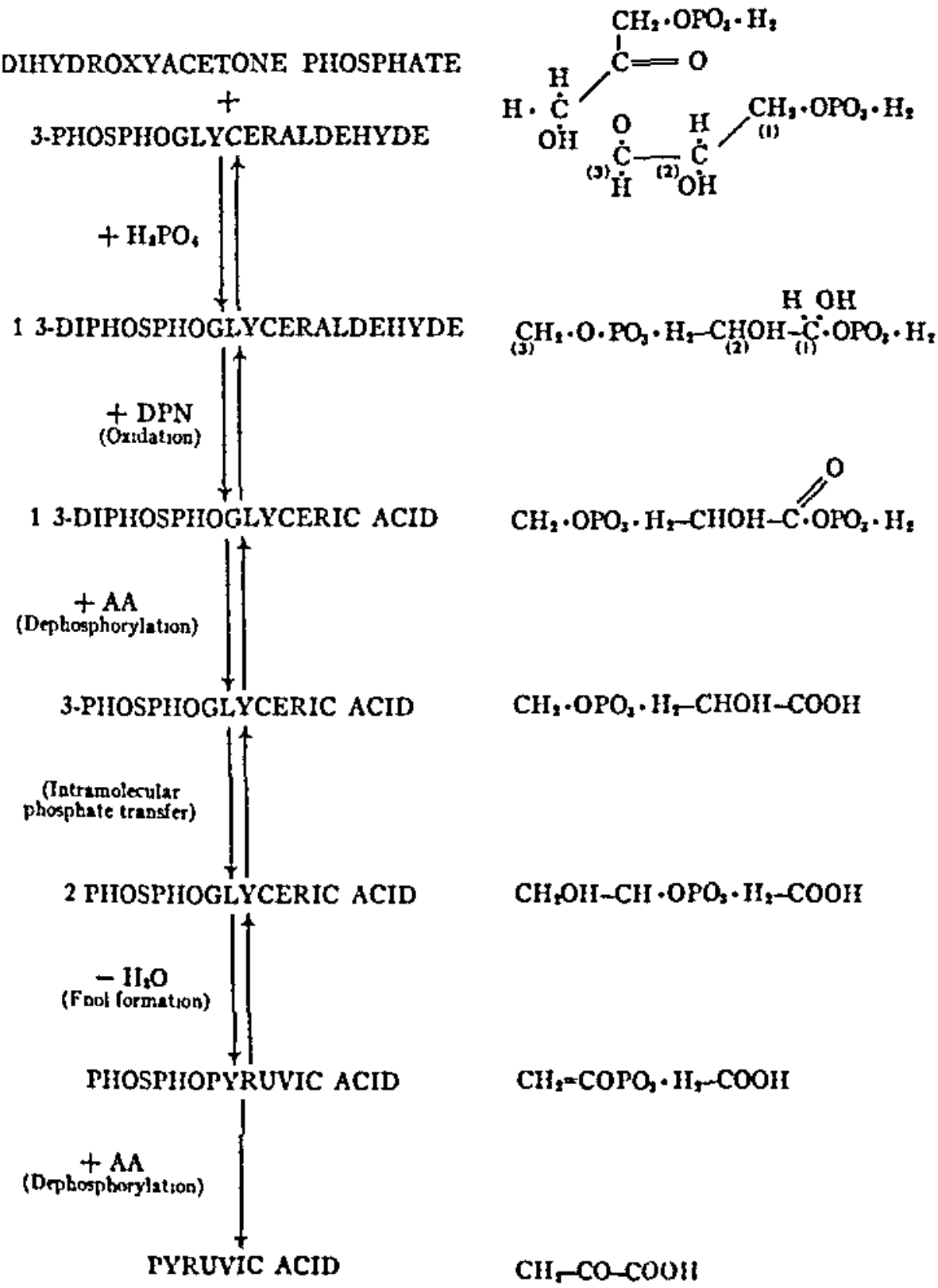


FIG 16 —*Continued*



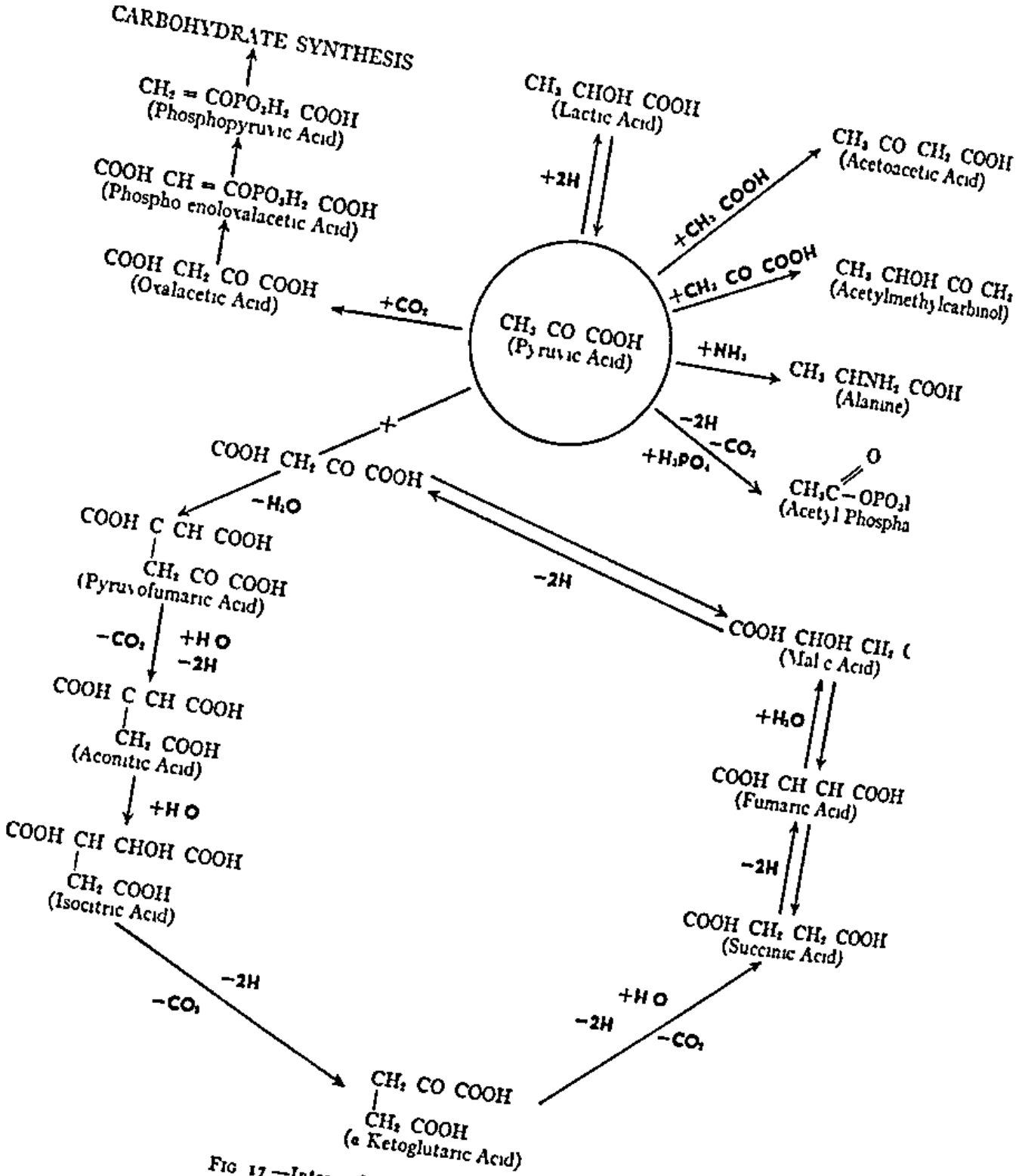


FIG 17 —Intermediary steps below pyruvic acid

THE FINAL COMMON PATHWAY OF METABOLISM

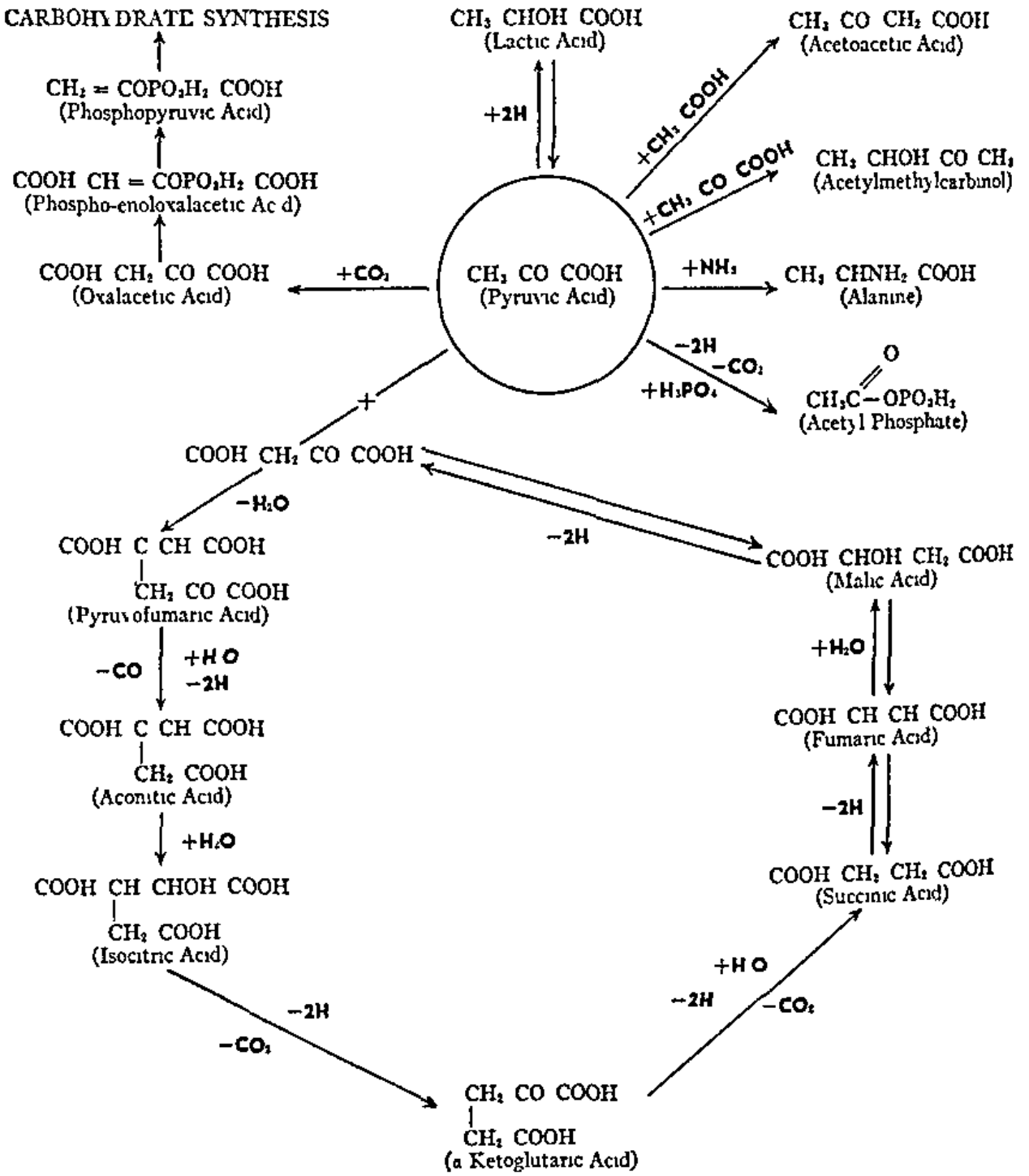
The tricarboxylic acid cycle may assume a significance far beyond its function in carbohydrate breakdown Many amino acids may be transformed directly or indirectly into one of the constituents of the cycle Conversely amination of the members of the cycle leads to the building of amino acids Furthermore the recent work of Wieland (13 14) and of Breusch (15) suggests that acetoacetic acid derived mostly from fatty acids may condense with oxalacetic acid to enter the same cycle Pyruvic and oxalacetic acids and their derivatives may therefore be regarded as forming the hub of the metabolic apparatus of the cell The cycle is probably the final common pathway for carbohydrate protein and fat as well as the locus for interconversions between the three foodstuffs (Fig 18) With this in mind much of the older controversy as to the interconvertibility of the foodstuffs (e g fat to carbohydrate) becomes pointless (see chaps xii and xiii)

ALTERNATIVE PATHWAYS

While the overwhelming mass of evidence supports the metabolic scheme outlined above there are strong indications that alternative pathways may exist For example in certain lower animal forms (fungi and bacteria) glucose may break down without the intercession of phosphorylations (16 17) Non phosphorylative glycolysis does not seem to be significant in vertebrate tissues so far as they have been examined (18) On the other hand there is indirect evidence that (under special circumstances in brain and skeletal muscle) the hexoses may be completely oxidized to CO_2 and H_2O without the intervention of the steps leading to pyruvate formation (19 20 21) It has been shown that complete oxidation proceeds unhampered in the presence of special inhibitors which stop glycolysis completely Although the alternate pathway has not been established there is some evidence to support the theory that hexose-6 phosphate may be oxidized directly (22 23) Figure 19 is a schematic representation of this hypothesis

CRITIQUE OF METABOLIC SCHEMES

The goal of the enzyme chemist is to separate the various catalytic systems to purify them to establish their chemical properties and to study the catalyzed reactions in a homogeneous medium *in vitro* This analytical outlook and procedure has enriched and will continue to add to our knowledge of the metabolic machinery of the cell in so far as the detailed properties of its parts are concerned However as in any other organized system the mere sum of the parts does not reveal the properties of the system as a whole In the living cell which is not a homogeneous system surface phenomena interaction between enzyme systems and other modifying influences may interfere with certain catalytic systems and promote others For example the rate of respiration of an intact cell is far smaller than the catalytic rate of the enzyme systems in the isolated state (8)



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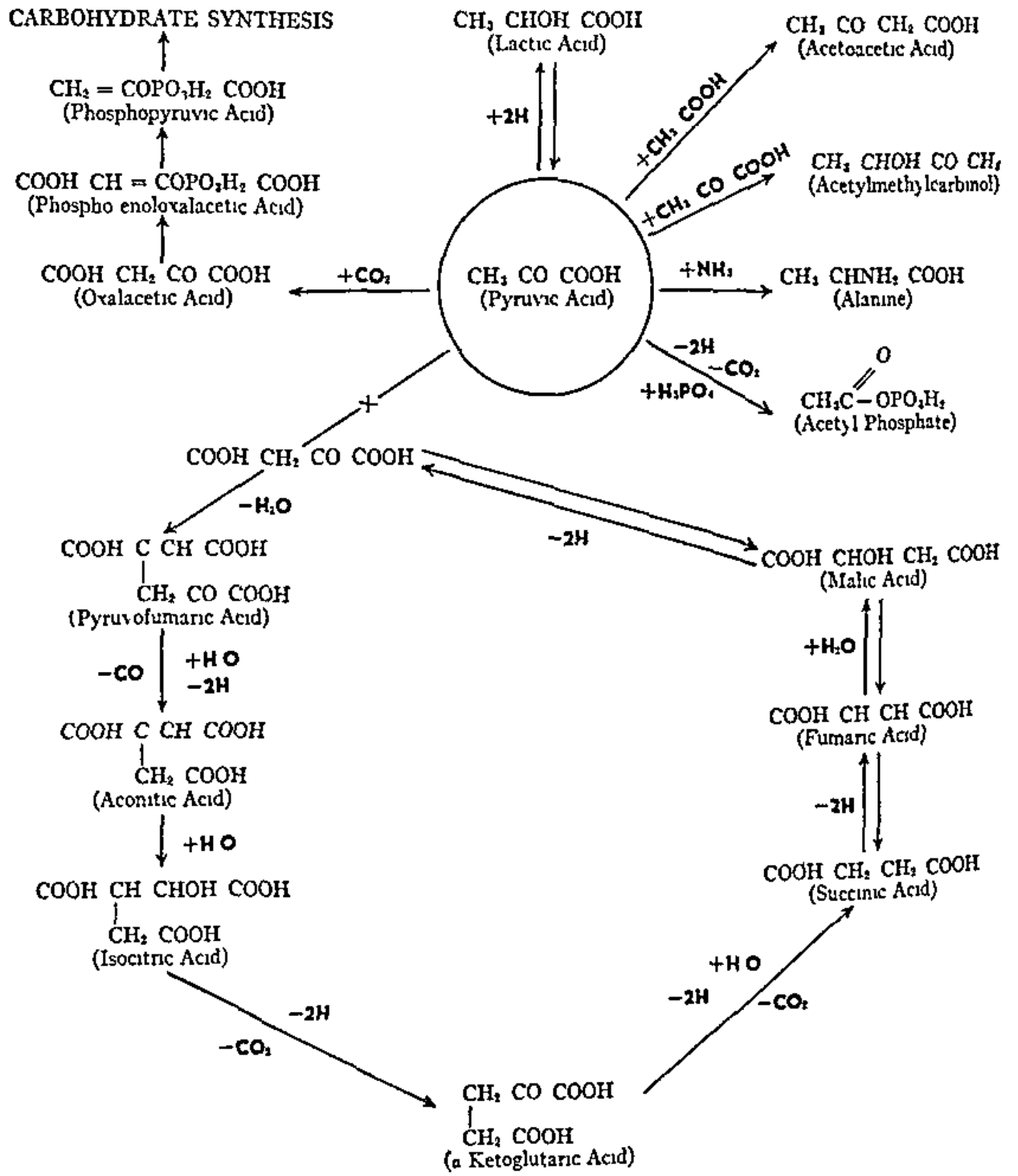

FIG 17.—*Intermediary steps below pyruvic acid*

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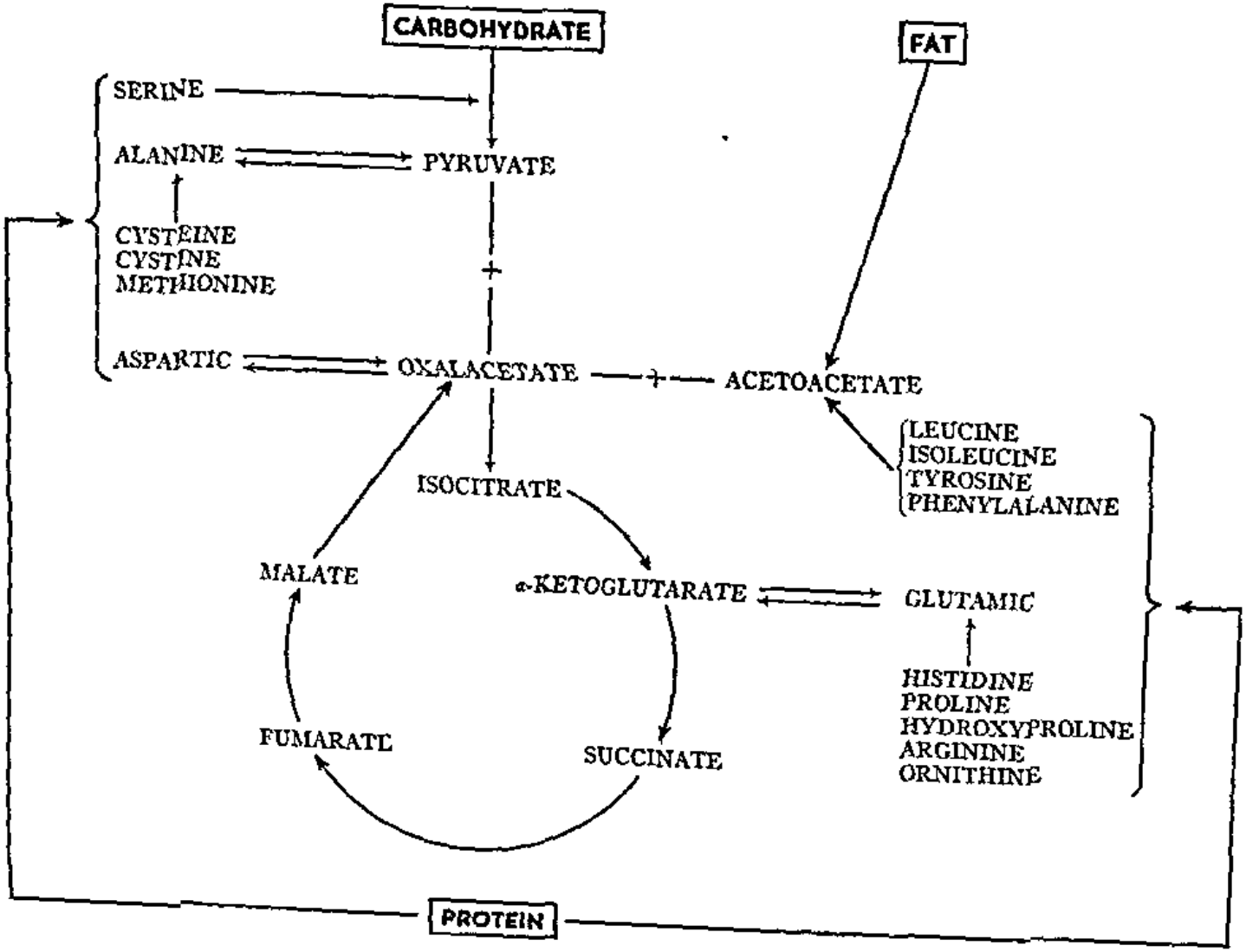

FIG 18.—*The final common pathway of metabolism*

An essential characteristic of the living cell is that its metabolism is regulated Of course the rates of reactions in the cell depend upon the relative concentrations of the activating proteins their coenzymes and the mineral elements (P Mg Fe etc) But many of the activating proteins in the carbohydrate scheme seem to depend for their activity upon sulphydryl groups (8 24) Oxidation of these groups leads to a loss of enzyme activity It is therefore probable that the glutathione of the cell serves as a regulator of activity for many systems

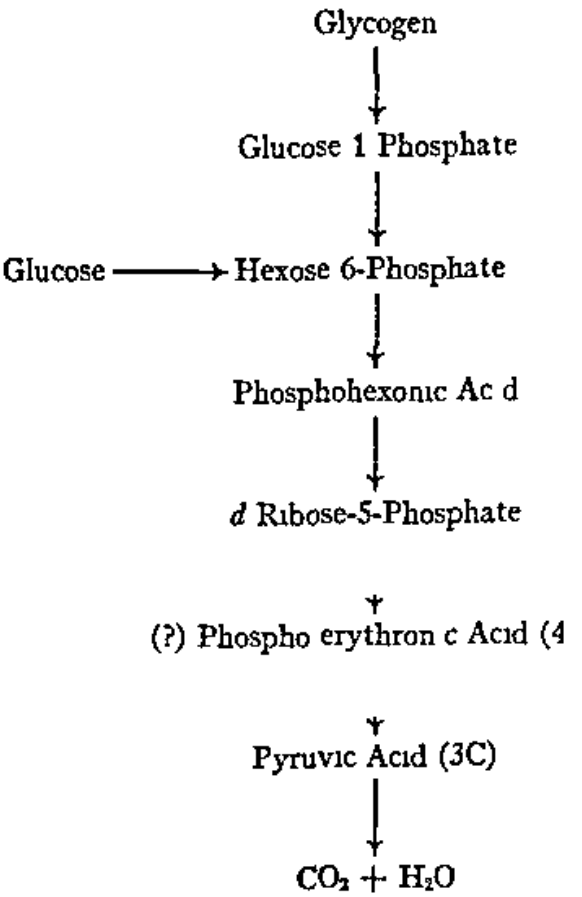

FIG 19 —Alternate pathway for carbohydrate dissimilation

Ever since Pasteur described the phenomenon it has been known that oxygen modifies the rate and direction of carbohydrate breakdown In the absence of oxygen most tissues rapidly break down glycogen or glucose to lactic acid in its presence carbohydrate breakdown is slower and little or no lactic acid appears The explanations of the mechanism of the Pasteur phenomenon are many and varied (25 26) In all probability there is no single mechanism for the total effect Oxygen may act by (1) removing lactic acid or its precursor (pyruvic) by oxidation to CO and H O or by resynthesis to carbohydrate (2) maintaining some enzyme system in an inactive state by keeping (indirectly) the protein sulphur groups in the S S state (3) inhibiting the usual pathway of breakdown of carbo

CHAPTER IV

THE LIBERATION AND TRANSFER OF THE ENERGY DERIVED FROM CARBOHYDRATE BREAKDOWN

THE total energy available from the complete breakdown of a molecule of a *foodstuff to* CO_2 and H_2O is inherent in its chemical structure The same amount of energy would be necessary to synthesize that foodstuff from CO_2 and H_2O Hence, the energy can be said to reside in the chemical bonds which link the atoms to form the complex molecule Different chemical bonds vary qualitatively and quantitatively Some bonds are more stable than others and are therefore less reactive A substance held together largely by such bonds is one from which the energy is less available than that from substances with unstable bonds Different chemical bonds also vary in the amounts of energy they represent In general, the high energy bonds tend to be the most unstable or reactive

According to the first law of thermodynamics, no more than the total bond energy of a substance can be derived from its complete breakdown, regardless of the pathway or the number of intermediate steps through which this occurs But common experience tells us that the form of the energy can be changed For instance, the living organism can transform the original chemical energy of a foodstuff into mechanical energy (e g , movement) Physiologists have long known that the body also produces electrical energy (e g , nerve impulses) When the chemical or bond energy of a substance is released, it raises the temperature of the medium in which the chemical reaction takes place We speak of this as a "transformation to heat " The body temperature of animals is maintained by a multitude of such reactions There are other reactions in which the converse is true, i e , energy has to be supplied from an outside source in order to make these reactions proceed In the laboratory we generally supply the energy in the form of heat and call such reactions "endothermic," in contrast to the "exothermic" reactions, which give off heat In the living organism, where temperatures are very constant, the energy necessary to make some reactions proceed is applied not as heat but as chemical or bond energy It is therefore more precise to characterize these reactions as "endergonic" and to speak of reactions in the living organism which yield energy as being "exergonic" (1)

It will be evident that the algebraic sum of the energies of the endergonic and exergonic reactions involved in the breakdown of a foodstuff to CO_2 and H_2O will be a positive sum of energy, *equivalent to the total bond energy* of the original

substance Under conditions in which this energy or any part of it has not been transmitted to objects outside the body, it finally appears and can be measured as body heat Upon this basis it has been possible to estimate total energy production (or requirements) of animals and man, under various conditions of rest and work, by measuring the total heat produced in suitable calorimeters By simultaneously measuring the total oxygen consumption of the organism it has also been possible to establish a caloric equivalent of the oxygen used The estimation of the rate of metabolism from the rate of oxygen consumption is known as "indirect calorime try "

It is obvious that neither the total heat produced nor the total oxygen consumed by the body during a given period of time can give any insight into the various forms through which the original energy has passed, nor can they indicate what bodily functions have been served The situation is analogous to the measurement of the heat produced by an electric light bulb made of opaque glass and of unspecified internal construction From the total heat given off one could calculate the amount of electric current which must have been used by the bulb, and perhaps also the amount of coal which it must have taken to produce that much electrical energy But one could not tell the amount of light present inside the bulb

SPECIFICITY OF ENERGY SOURCE

It has been customary to speak of metabolic energy as if it were an undifferentiated reservoir of power serving all cellular functions in a non specific way However, recent evidence has indicated that this is not so Particular functions require particular sources of energy Indeed, they may require that the energy be derived from a specific chemical reaction This is not surprising when one compares the situation with that which obtains with regard to internal combustion engines If one takes a quantity of gasoline and a quantity of fuel oil of the same caloric equiv alent, the former could be transformed into useful mechanical energy by a motor car but not by a Diesel powered truck, while the fuel oil would be useful in the truck and not in the car A striking example of the specificity of fuel in the living organism is the essential nature of glucose for the activity of the central nervous system When isolated brain tissue is studied *in vitro* by the Warburg technique, it can readily be demonstrated that its oxygen consumption (energy production) can be as well maintained at the expense of pyruvate or succinate as by the use of glucose (2, 3, 4) Nevertheless, in the intact living animal the brain evidences serious functional difficulty as soon as the blood sugar level falls below about 40 mg per cent Apparently, the normal irritability of the central nervous system depends upon chemical energy derived from glucose This function cannot be main tained at the expense of the energy derivable from lower intermediary substances (5, 6, 7)

THE ENERGY TRANSFER FUNCTION OF PHOSPHATE GROUPS

It is now known that the various phosphorylations which occur throughout the dissimilation of carbohydrate are the means by which the energy liberated from oxidative steps is prevented from being dissipated as heat and is held or built up for use in endergonic reactions (8, 9) Different phosphorylations carry different amounts of energy and are, therefore, suitable for motivating different kinds of endergonic reactions (9) According to the amount of energy transferred, we speak of high energy or of low-energy phosphate compounds or bonds Inorganic phosphate is of course, at the lowest energy level The high energy phosphate bonds (10,000–12,000 cal/mole) are present in such compounds as adenosine triphosphate (ATP), creatine phosphate, acetyl phosphate, phosphopyruvic, etc As an example of how a high energy phosphate bond performs its function, let us consider the manner in which glucose is transformed into glycogen, a carbohydrate of higher potential energy than its precursor A superficial representation of the chemical steps between glucose and glycogen might be written as follows

$$\text{Glucose} \rightarrow \text{Glucose-6 phosphate} \rightleftarrows \text{Glucose-1 phosphate} \rightleftarrows \text{Glycogen}$$

From an energetic standpoint this reaction by itself is impossible, since it requires the addition of energy to raise glucose to the energy level of glycogen, and there is no indication whence this energy is derived These reactions can be made to proceed *in vitro* by adding certain protein enzymes and ATP (10, 11, 12) The energy which drives the reactions is derived from the high-energy phosphate bonds in the ATP The latter loses its labile phosphates, becoming adenylic acid in the process

Since the amount of ATP present in living cells is limited, the more complete story of the series of reactions in the living organism must include the manner in which adenylic acid is rephosphorylated to ATP This may occur in more than one way, but an important means is through the energy liberated by the oxidation of 3 phosphoglyceraldehyde to 1 3 phosphoglyceric acid (13) The energy made available by the oxidation of the aldehyde to the acid is incorporated in a high energy phosphate bond in the acid In a sense, therefore, we may say that the oxidative energy has raised the inorganic phosphate involved in the reaction to a higher energy level (9) The motivating power of the chain of events having thus been applied, the cycle proceeds in the manner graphically illustrated in Figure 20 It may be seen that the ultimate use of the original oxidative energy, applied through ATP, is to raise the lower energy foodstuff (glucose) to the higher-energy storage product (glycogen) At the latter point the phosphate group involved in the series of reactions is divorced from the substrate and may re-enter the cycle at the beginning

The raising of glucose to the energy level of glycogen is only one of the functions which ATP performs Indeed, the reversible systems AA $\rightleftarrows$ ADP $\rightleftarrows$ ATP seem to

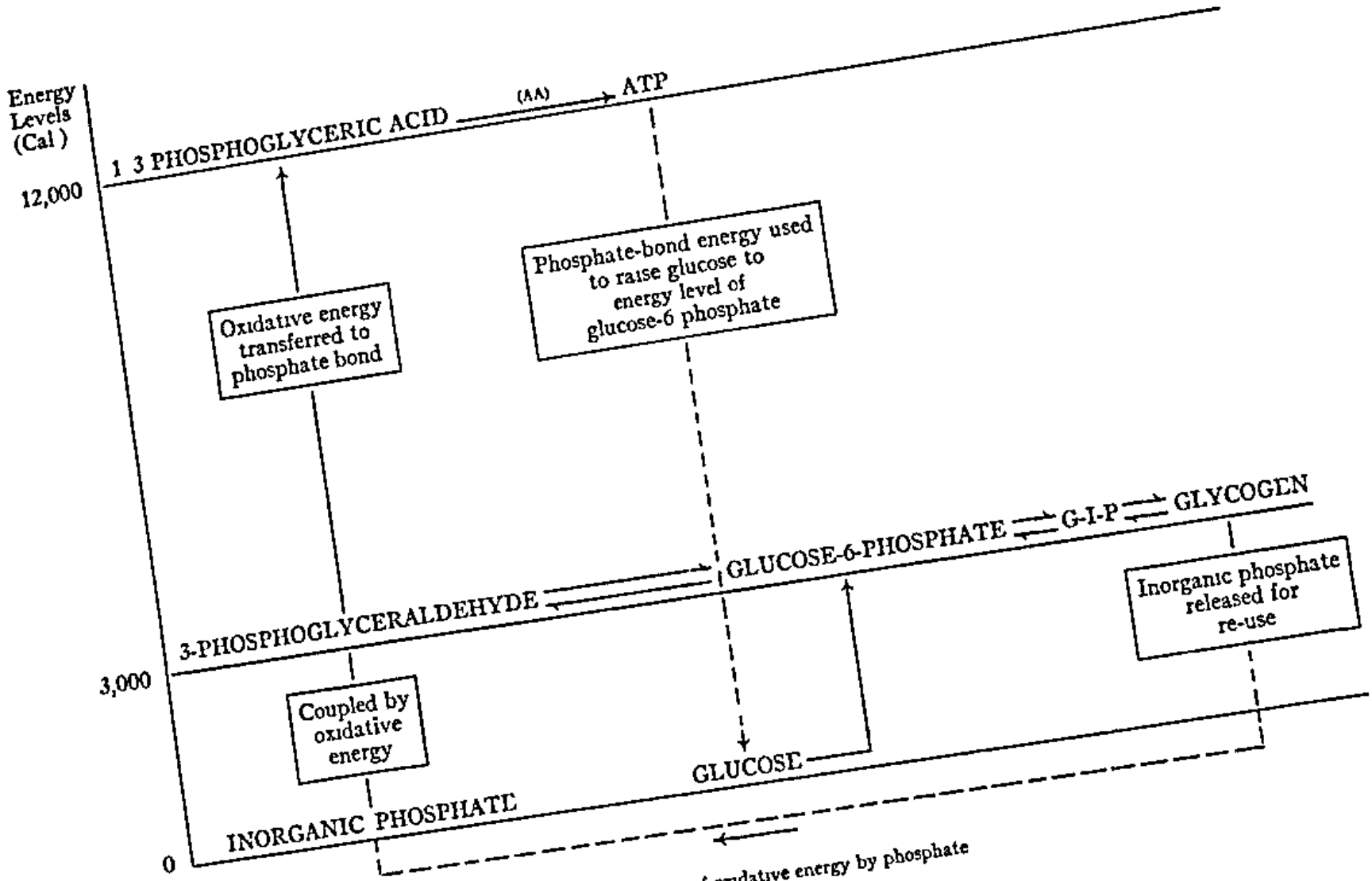

FIG 20.—Transfer of oxidative energy by phosphate

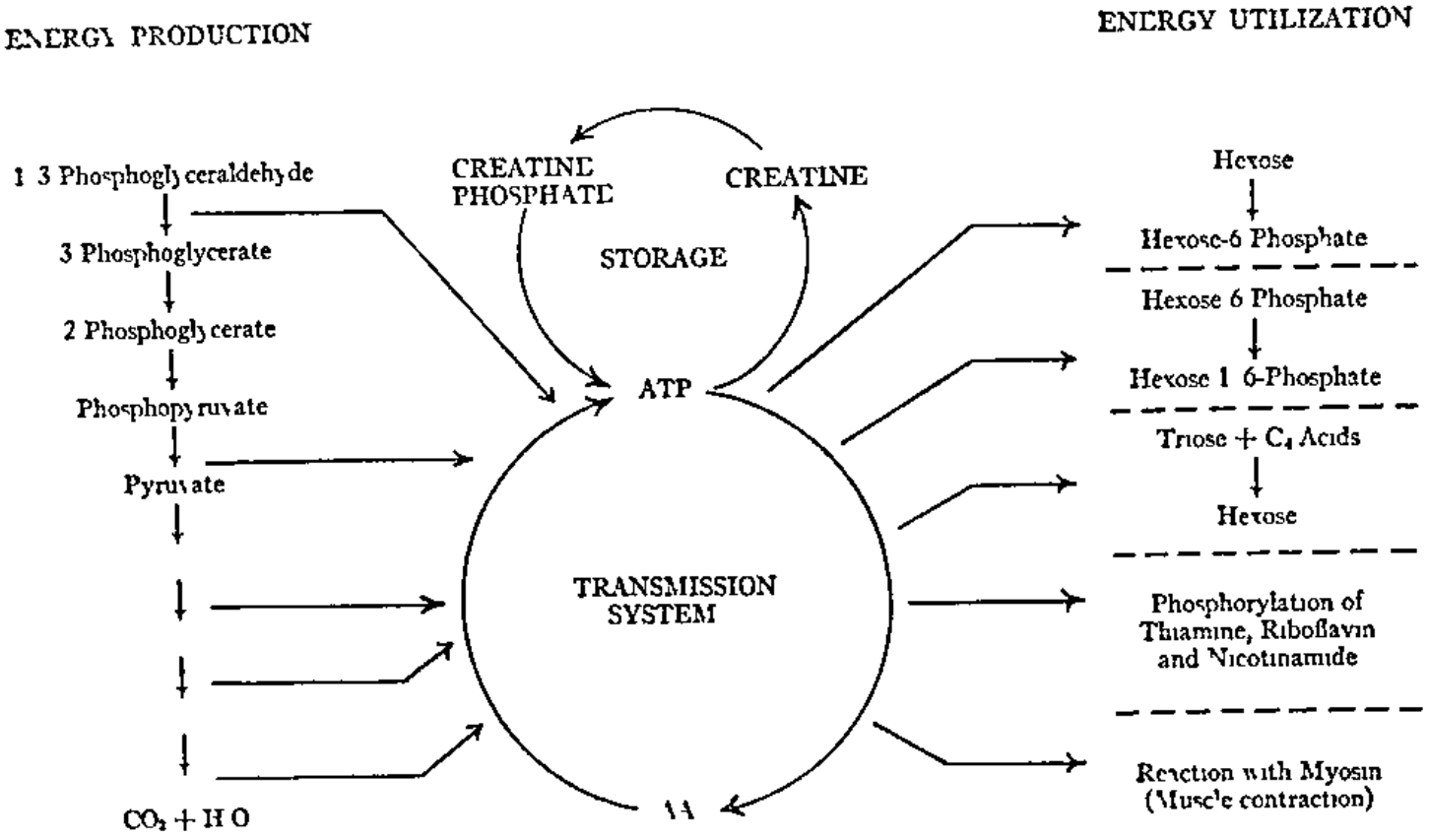

FIG 21 —Central position of the adenylic system in energy transfer

be the central mechanisms for energy transfer from exergonic to endergonic reactions in carbohydrate metabolism Figure 21 summarizes their relationships to all the known energy cycles

There has been considerable doubt as to the place of the Creatine ⇌ creatine phosphate reaction in the general scheme Because of its high energy phosphate bonds, some authors have ascribed to creatine phosphate a role similar to that indicated for ATP It now seems more likely that the latter is not the case but that creatine phosphate acts as an emergency store of high energy phosphate bonds This store is built up at times when the AA ⇌ ATP systems are producing an excess of energy over the requirements of the moment and is broken down when the ATP mechanisms cannot supply energy as rapidly as is required Thus, creatine phosphate stands in the same relationship to the storage of energy as glycogen stands in relation to the storage of carbohydrate substrate

Finally, it should be noted that the transference of energy by means of phosphate bonds accounts for the ready reversibility of most of the reactions of carbohydrate metabolism (8, 9, 14) This is because the energy which is yielded by the substrate remains "attached" to the product of the reaction and is therefore not lost from the system For example, the hydrolytic splitting of glycogen by amylase produces glucose and liberates energy as heat The analogous phosphorolytic cleavage of glycogen in the body (see Fig 12, p 38) produces glucose 1 phosphate, with the energy retained in the phosphate bond Hence, no outside energy is necessary to reverse the process (8, 15)

Regarded as a whole, the pattern of energy interchange in carbohydrate metabolism is by no means as complicated as a consideration of the details might lead one to believe The general principle may be compared to that employed in the mining and use of coal Figure 22 is a diagrammatic representation of the analogy, in which various features are labeled with their metabolic counterparts The essential features are the investment of a certain amount of energy to procure large amounts of an energy substance (coal in the mine shaft or glucose in the body), the raising of the energy substance to a higher energy level (the coal pile on the surface, or glycogen in the body), the conversion of the energy substance into another form of energy (running the electric generator from a steam engine fired by coal, or phosphorylation in the body), the use of the more convenient form of energy for the transfer of power to places where it can be used for special purposes (use of electric power for communication, transportation, etc , or the use of phosphorylative energy for muscle contraction [8], nerve conduction [16], intestinal absorption [17] renal reabsorption [18], calcification [19], sperm motility [20], etc), and, finally, the use of some of the energy derived from the energy substance to obtain more of the energy substance (use of some of the electrical energy made from the coal for the purpose of mining more coal, or the phosphorylation of glucose in the body)

Since we do not, as yet, possess a detailed knowledge of all or most phosphate-energy transfer reactions, the efficiency of this mechanism can be judged only approximately It has been shown that, during the complete dissimilation of 1 mol of glucose to CO_2 and H_2O, from twelve to twenty-four high-energy phosphate bonds are formed (21, 22, 23, 24). The energy content of these phosphate bonds is, therefore, 144,000–288,000 cal Since 1 mol. of glucose going to CO_2 and H_2O yields 673,000 cal , the energy transferred by means of phosphate bonds represents about 21–42 per cent of the total It is interesting to compare these figures with that of the efficiency of muscular work, which is generally considered to be about 30 per cent

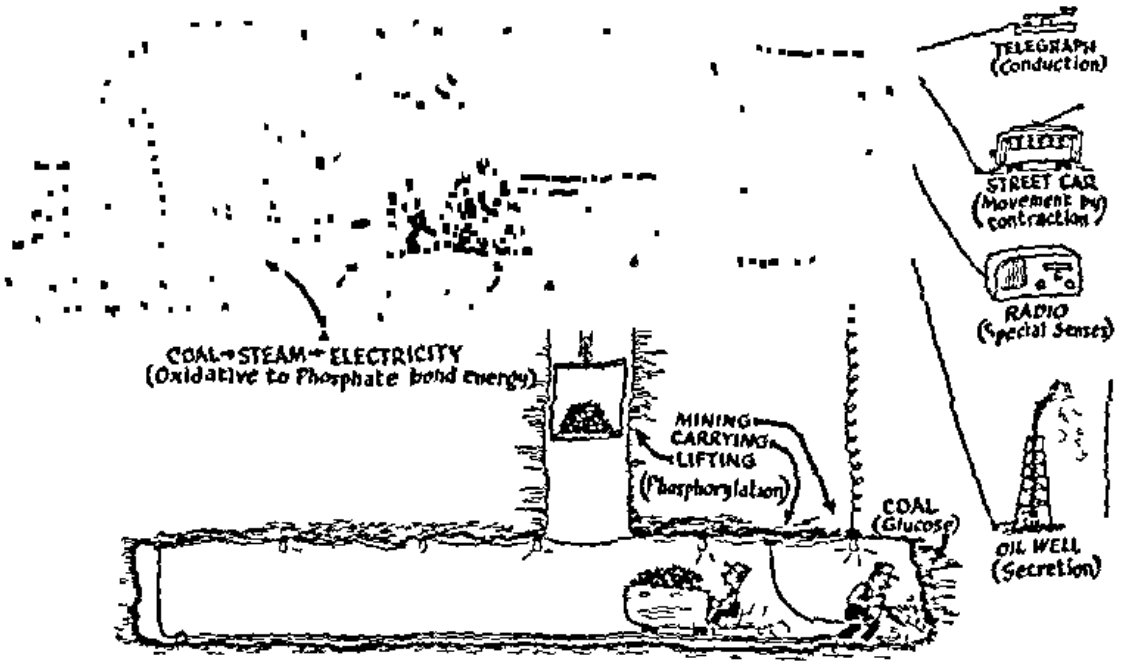

FIG 22.—Analogy to the liberation, transfer, and utilization of metabolic energy

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CHAPTER V

THE USE OF ENERGY FOR MUSCULAR CONTRACTION

IN THE previous chapters we clarified our concepts as to the nature of the energy derived from carbohydrate and the manner in which this energy is transformed and made available for the various uses to which it is put The actual results of the expenditure of useful energy in the body may be observed in terms of muscular contraction, glandular secretion, nervous activity, etc It remains to consider in detail how the very real but invisible energy of the foodstuff is translated into tangible physiological performance Muscular contraction will serve as the best example for this purpose This is partly because more is known about this function than about any other and also because it is quantitatively the most important energy outlet

Skeletal or voluntary muscle comprises approximately 50 per cent of the body weight It consists of 75–80 per cent H_2O and 20–25 per cent solids The *dry weight* of the muscle is partitioned as follows (omitting lipoids and minerals)

75–80 per cent proteins
2 5–5 0 per cent glycogen
2 0–3 0 per cent creatine phosphate and free creatine
1 0–1 5 per cent adenosine phosphates
1 0 per cent other phosphorylated products of carbohydrate metabolism

It may be seen that protein is the chief structural component of this tissue But it must be remembered (as pointed out in chap II) that most, if not all, of the proteins of the living cell function as enzymes as well as structural elements Next to protein in quantitative importance are the two storage products, glycogen (the fuel reserve) and creatine phosphate (the more readily available energy reserve) Adenylic acid and its phosphorylated forms, which constitute the active phosphorylating system of the muscle, represent a small but significant fraction of its bulk The remainder of the muscle is composed of a number of intermediate metabolites which are caught in transit

THE PHYSICAL NATURE OF MUSCLE CONTRACTION

The contractile element responsible for the shortening and elongation by which muscle performs its physiological function is myosin, one of its proteins (1, 2) Myosin is present in the form of elongated, threadlike structures called ' muscle

fibrils " These are microscopic in size A bundle of fibrils, composed of large numbers in parallel formation, constitutes a muscle fiber The gross structure of a muscle is composed of aggregates of fibers The myosin of the muscle fibrils represents approximately half of the total muscle protein

Both in its shape and its elastic properties the myosin fibril resembles a rubber band (3, 4) It is not unique in this for keratin and wool are proteins of the same type But myosin differs from these other proteins in having an *internal* mechanism by which it is stretched The contraction of a fibril is due to the release of this mechanism and to the fibril's recoil to a neutral position X ray diffraction studies have indicated that the internal configuration of the myosin molecule, in its stretched and collapsed states, changes as shown in Figure 23 (4)

It will be noted that a relatively new and unorthodox conception of muscle states has been introduced in the preceding paragraph It has been customary to speak of a resting muscle as "relaxed" and of a working muscle as "contracted " As these terms imply, it was formerly thought that the energy expended in work was applied in bringing about the shortening or contraction of muscle, while relaxation was merely the result of the cessation of the expenditure of contractile energy The newer evidence, that the resting muscle resembles a stretched elastic band, necessarily reverses the locus of application of energy The external force exerted by the contracting muscle is a result of the recoil of its stretched fibrils, while the metabolic energy is applied to return the collapsed elastic members back to their original state of stretch

THE CHEMICAL EVENTS ACCOMPANYING MUSCLE CONTRACTION

The first chemical changes to be related to the change in the physical state of the muscle during contraction were the breakdown of glycogen and the appearance of lactic acid (5, 6) Lundsgaard's demonstration that contraction of muscle was possible in the presence of iodoacetate, which prevented lactic acid formation, forced the abandonment of this hypothesis He further demonstrated a parallelism between the breakdown of creatine phosphate and the energy liberated by the iodoacetate treated muscle This led to the hypothesis that the immediate source of energy for muscular contraction was the breakdown of creatine phosphate, while the glycolytic process served to resynthesize the creatine phosphate from its split products (7, 8, 9)

The current conception of the means by which metabolic energy is applied to the muscle fibrils was initiated by the work of Lohman, who showed that adenosine triphosphate (ATP) was necessary both for glycolysis in muscle and for the synthesis of creatine phosphate (10, 11) This was followed by Parnas' demonstration that the breakdown of creatine phosphate merely served to supply phosphate for the conversion of adenylic acid to ATP, without the liberation of energy, while the subsequent breakdown of the ATP actually supplied the energy for contraction

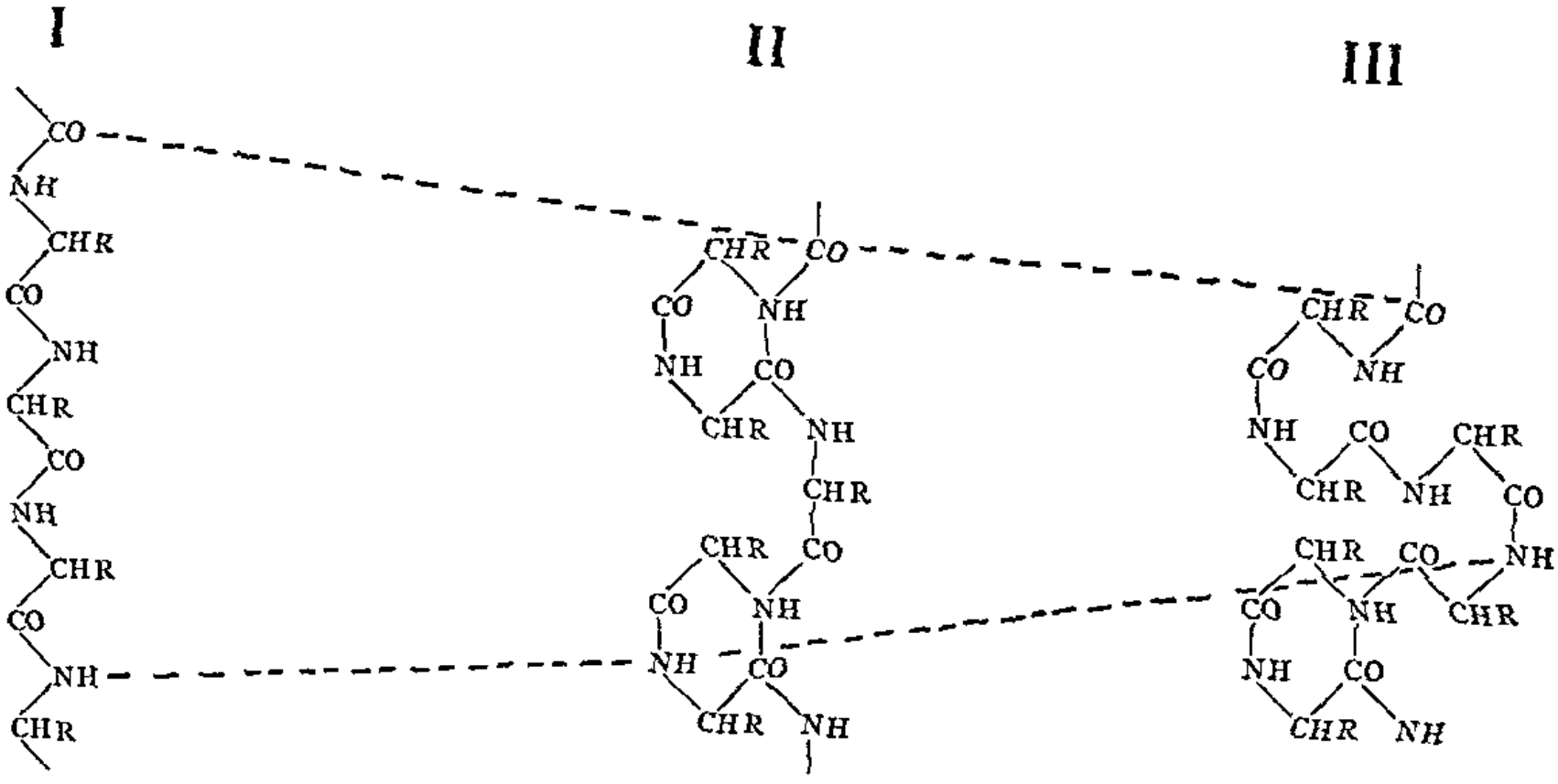


FIG 23.—Stretched and contracted forms of the myosin molecule. *I* artificially stretched to limit of extensibility by mechanical means. *II* as the myosin molecule is thought to exist in relaxed state *in vivo*. *III* as it is thought to exist in contracted state *in vivo*. R represents the various amino acid side-chains (Astbury [3]).

and the phosphate for glycolysis (12 13) The glycolytic reactions in turn provided the energy for the resynthesis of both creatine phosphate and ATP

It may be seen that as our knowledge of the subject has developed the breakdown of glycogen to lactic acid has been gradually relegated to a secondary process with a restorative function As a matter of fact the most recent evidence indicates that under ordinary physiological conditions glycogen breaks down without the appearance of lactic acid at all (p 49) When the rate of oxygen supply to the muscle is adequate for the rate of glycogen breakdown pyruvic acid is oxidized completely and none of it is reduced to lactic acid Under these conditions oxidative steps above and below pyruvic acid supply energy for the rephosphorylation of ATP and thus maintain the metabolic cycle in the absence of lactic acid It is only when the oxygen supply is inadequate (as it was in most of the experiments of the earlier investigators) that lactic acid appears This occurs because pyruvic acid partially substitutes for oxygen by becoming the hydrogen acceptor from reduced DPN and in so doing is itself reduced to lactic acid

In a sense therefore the formation of lactic acid by muscle is merely an emergency mechanism enabling muscular contractions to occur for a short time despite a lack of oxygen This may be useful at the beginning of sudden or severe muscular work to tide the muscle over a period of circulatory adjustment that is while the blood supply is changing from the slow rate adequate during rest to the more rapid rate necessitated by the exertion (14) It also enables the muscle to exert a relatively tremendous effort for a short space of time at a rate with which the maximal rate of oxygen supply could never cope The lactic acid which accumulates during such an effort is reoxidized to pyruvic acid when the exertion is over This process may be regarded as the repayment during comparative leisure of an energy debt contracted under stress

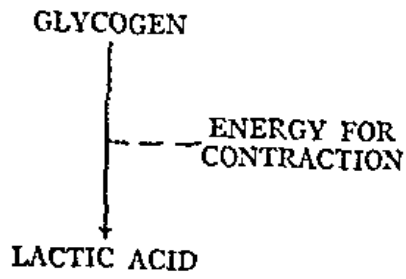
Figure 24 graphically illustrates the development of our concepts concerning the sequence of chemical events which occur during muscular contraction

Although it is out of place here to attempt an analysis of conflicting data in respect to the chemistry of muscular contraction (as it occurs *in vivo*) it should be pointed out that the work of Sacks (15) and of others (16) indicates that the scheme as given in Figure 24 may have to be modified to account for the sequence of chemical events in the living intact muscle

THE CONNECTION BETWEEN THE PHYSICAL AND CHEMICAL EVENTS IN MUSCLE CONTRACTION

Thus far we have merely described the chemical events which occur coincidentally with muscular contraction It remained for Engelhardt (17 18) to demonstrate the direct causal link between the chemical reactions and the change in the physical state of the myosin In so doing he confirmed the dominant position of ATP in the chemical processes as well as the previously described physical nature

I



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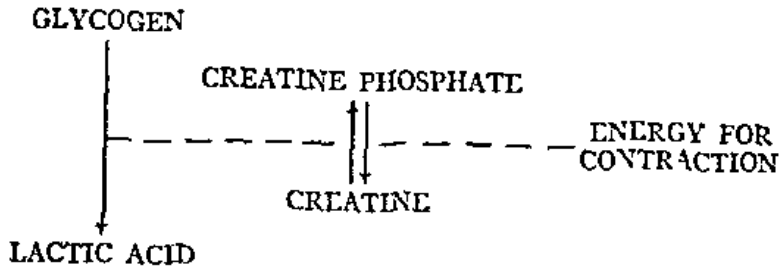

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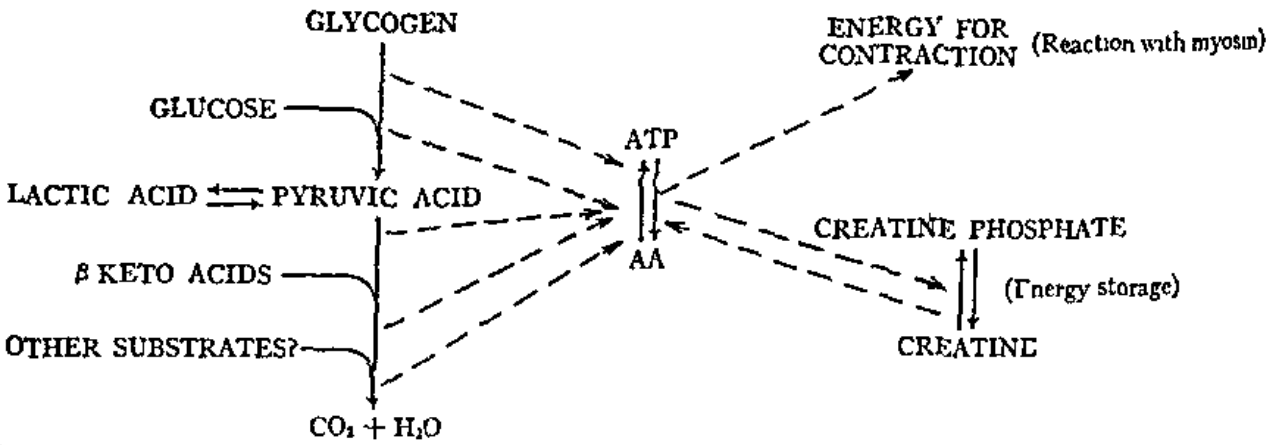

FIG 24.—Development of concepts of the chemistry of muscular contraction *I* Hopkins-Meyerhof hypothesis, Lundsgaard modification, *III* current scheme indicating the secondary role of lactic acid, the central position of adenylic system, the energy storage function of creatine phosphate, and the use of the energy in ATP by myosin

of contraction (i e , the recoil of a stretched fiber) By injecting a thin stream of a purified myosin preparation into water, Engelhardt (18) was able to make threads of myosin analogous to muscle fibrils and possessing similar elastic properties When suitably weighted and suspended in water, these myosin threads were not affected by the presence of the various mineral and organic substances normally found in mammalian muscle But the addition of ATP to the water was followed by a definite increase in the length of the threads which could be reversed by flushing away the ATP

Szent Gyorgyi and his co workers (19) confirmed Engelhardt's work and ex tended it into a more complete analogy of *in vivo* muscular contraction They found that a purer preparation of myosin than that used by Engelhardt would not form threads when injected into water But when another muscle protein (which they named ' actine) was added to the myosin, the compound behaved like Engelhardt's preparation They named this complex ' actomyosin" and found that threads formed from it could be made to extend or contract at will by varying the proportions of ATP potassium, and magnesium added to the water in which they were suspended

The extremely simple conditions of Engelhardt s and Szent Gyorgyi's experi ments leave no doubt that ATP is the prime agent responsible for the stretching of myosin fibrils that is preparatory to muscle contraction The peculiar appropriate ness of ATP for this purpose lies in the fact that it had previously been shown that myosin is the enzyme which splits ATP $\rightarrow$ ADP + P_0 (20, 21) For the time be ing, we may therefore accept the current scheme shown in Figure 24 as represent ing the cycle of events by which metabolic energy derived from the utilization of carbohydrate is transferred by ATP and applied to the contractile elements of the muscle The train of reactions is such that both the original physical state of the muscle and the original amount of ATP are restored subsequent to contraction

It is evident from our present conception that any metabolic intermediate which can supply the energy necessary to restore AA to ATP can serve as a fuel of muscular exercise This applies to α and β ketoacids derived from protein and fat as well as to carbohydrate derivatives (see Fig 18, p 54)

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PART II

INTRODUCTORY PHYSIOLOGICAL CONSIDERATIONS

CHAPTER VI

NATURE AND OCCURRENCE IN THE TISSUES OF MATERIALS IMPORTANT TO CARBO HYDRATE METABOLISM

IN THE previous chapters we discussed the ultimate use of carbohydrate by the effector organs and the manner in which the chemical energy of the food stuff is liberated and applied to physiologic functions It will readily be appreciated that this knowledge however fundamental and important, is only a small part of the larger body of information with which it must be integrated in order to understand carbohydrate metabolism in the living organism As opposed to chemical reactions in the laboratory, an essential characteristic of metabolic functions *in vivo* is that they are finely regulated processes adjusted in each organ and tissue to the needs of the body as a whole It is with the complex series of actions and interactions between tissues and organs subject to intrinsic endocrine and nervous regulation that we must now deal But before beginning our account, it will be useful to describe in some detail the nature and occurrence in the tissues of various substances which are important to carbohydrate metabolism—substances which have been briefly mentioned in the preceding chapters and which we shall meet again in subsequent chapters

GLUCOSE

Glucose is the chief and for practical purposes the only, transport form of carbohydrate Carbohydrates enter the blood from the gastro intestinal tract largely as glucose In the post absorptive state, glucose is the carbohydrate which the liver supplies to all the other tissues of the body For these reasons the level of glucose in the blood is normally higher than in any other tissue or fluid of the body

The average normal level of glucose in the blood does not vary appreciably with the species of animal In most mammals it is very similar, ranging from 60 to 80 mg per 100 cc of whole blood It has been customary to express these amounts as '60–80 mg per cent' Strictly speaking, this is incorrect, for whole blood is not homogeneous nor is it of the same specific gravity as water Nevertheless, with the reservations noted we shall make use of this shorthand designation of concentration for the sake of convenience

The blood sugar levels reported by different observers depend to a certain extent, upon the methods employed for chemical analysis Glucose is an aldohexose

(see Fig 25) in which the aldehyde group on the first carbon atom acts as a reducing agent Hence, the most practical and most commonly used chemical methods for determining glucose are procedures in which a metallic ion in the oxidized state (usually copper) is reduced by the sugar Such methods were devised by Bertrand (1), Folin (2), Hagedorn (3), Somogyi (4) and many others (5, 6) They differ from each other chiefly as regards the means by which reducing substances other than glucose are removed from the reaction To the extent that these means differ in efficiency, there are differences in blood sugar values reported from various laboratories For example the range of normal values quoted for mammals is obtained by the Somogyi modification of the Shaffer Hartman method When the Folin Wu method is used, a range of from 80 to 120 mg per cent is obtained Somogyi has shown (4, 7) that his method of precipitation removes virtually all the non carbohydrate reducing substances (chiefly glutathione), hence, the results

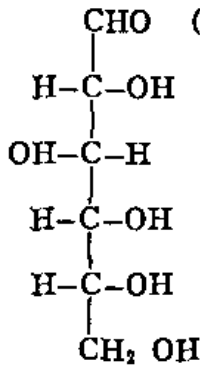

Fig 25 —Glucose

obtained by using his method are sometimes referred to as values for ' true blood sugar

When the level of sugar in a sample of whole blood is 100 mg per cent, the concentration of sugar in the plasma of the same blood is about 115 mg per cent (8, 9) This difference is due to the fact that the sugar is not equally distributed between the blood plasma and the red blood cells (There is an equal distribution of glucose between the blood plasma and the water phase of the red blood cells [8, 10]) The precise difference between the whole blood sugar and the plasma sugar in a given instance will depend upon whether or not the normal number of red blood cells per unit volume of blood is present

Because the peripheral tissues are constantly removing sugar from the blood samples of arterial or capillary blood will show a level of sugar a few milligrams per cent higher than that of simultaneously drawn samples of venous blood (11, 12) This so-called A–V difference varies with the existing rate of utilization of sugar and also depends upon the rate of blood flow through the tissues at the time of sampling (13, 14) It is obvious that, if the rate of sugar utilization were con

stant, a doubling of the rate of blood flow would result in a diminution of the A–V difference to half its former value Neglect of this simple consideration has given rise to some confusion in the literature (14, 15)

Table 7 lists the range of sugar values reported in various fluids and secretions of the body Being a crystalloid of small molecular weight, glucose diffuses readily out of the blood stream into all other body fluids Tissues like liver or skeletal muscle are composed of at least two phases, namely, the tissue cells and the fluid filling the interstices between them (extracellular fluid) The sugar in the blood plasma would rapidly equilibrate with the sugar in the extracellular fluid were it not for the constant withdrawal of sugar from the latter by the cells The actual level of glucose in the extracellular fluid is therefore a few milligrams lower than that in the blood plasma But analysis of normal whole muscle for its glucose con tent (using the proper precautions to prevent glycolysis) usually yields a range of

TABLE 7

GLUCOSE CONTENT OF BODILY FLUIDS

Fluid	Glucose Content (Mg per Cent)
Whole blood	60– 90
Blood plasma	70–110
Lymph	70–110
Cerebrospinal fluid	40– 70

values between 30 and 60 mg per cent This, of course means that the cells them selves contain much less free sugar than does the extracellular fluid An estimate of the amount of glucose actually present within the tissue cells may be made by de termining the amount of extracellular fluid and calculating the intracellular sugar from the sugar content of the whole tissue

Normal urine contains a small amount of glucose An average adult human ex cretes from $\frac{1}{2}$ to $\frac{3}{4}$ gm in the approximately 1,500 cc of urine excreted in 24 hours (16 17) In clinical medicine such urine is termed "sugar free," because the rou tine methods for the qualitative detection of sugar are not sufficiently sensitive to indicate its presence in this concentration That the concentration of glucose in normal urine is far below that occurring in other body fluids is not because the membranes of the kidney are less permeable to sugar The kidney glomerulus ac tually passes a filtrate containing glucose in the same concentration as is present in blood plasma (18) But this filtrate is then subject to the action of the cells of the kidney tubules which reabsorb most of the sugar in it (19, 20)

The process by which the kidney tubules reabsorb glucose depends upon phos-phorylating mechanisms (21, 22) Inhibition of the latter by the glucoside phlor hizin prevents the reabsorption of the sugar and results in so-called "phlorhizin diabetes" (23, 24) Abnormal amounts of sugar also appear in the urine whenever the blood sugar level is raised to such heights that the amount of glucose filtering

through the glomeruli exceeds the phosphorylative capacity of the tubules The critical level at which this begins to occur is usually about 180 mg per cent and is often referred to as the kidney threshold for glucose (20 25)

GLYCOGEN

Glycogen in the animal body is similar in form and function to the starch in plants It is a polymer consisting of many glucose molecules joined to each other in the manner indicated in Figure 11 (p 37) The C O–C– linkage between adjoining glucose molecules is known as the glucosidic linkage It is here that the glycogen molecule splits with the introduction of a phosphate group (see p 35) The distribution and particular significance of the glycogen in various tissues is discussed in a number of places throughout this book (see p 9) and will not be repeated here

Glycogen when isolated in the laboratory is a stable compound But in the presence of the tissue enzyme systems it breaks down very easily For this reason the glycogen content of a dead tissue gives no indication of its content during life and accuracy of estimation is not assured even when tissue is removed from the living organism This is especially true when any degree of anoxia is allowed to occur while the tissue is being removed for analysis or in the case of muscle when twitching of the muscle fibers is induced by careless handling A probable reason for the susceptibility of glycogen to anoxia is that the active form of glycogen phosphorylase (see p 36) contains SH (reducing) groups Hence any degree of oxygen lack would tend to keep the enzyme in its reduced form and would therefore favor the phosphorylation and breakdown of glycogen Another reason may be the rapid appearance of inorganic phosphate during oxygen lack This favors glycogenolysis by shifting the equilibrium of the following equation to the right

$$\text{Glycogen} + P_o \rightleftharpoons \text{Glucose-1 phosphate (26)}$$

The standard method for glycogen estimation in tissues depends upon the fact (discovered by Claude Bernard and put to practical use by Pfluger) that hot concentrated potassium hydroxide destroys all carbohydrates except glycogen As described by Good Kramer and Somogyi (27) the method is accurate and relatively simple once the tissue is dissolved in the alkali The difficulty consists in removing and transferring the living tissue into the alkali before any significant amount of glycogen disappears Fairly good and consistent results may be obtained by anesthetizing the animal with an anesthetic (such as amytal or pentobarbital) which does not itself tend to break down glycogen The tissue to be stud

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taining hot alkali But the techn qu by which

tent of a tissue is most nearly determinable is the following The animal is anesthe

tized, and the tissue prepared as above The tissue is then frozen *in situ* by the use of liquid air or crushed CO_2 ice It is removed and weighed in the frozen state and immersed in the hot alkali

LACTIC ACID

When the body is at rest and in the post absorptive state, the lactic acid con tent of the blood ranges between 10 and 20 mg per cent (28, 29) The lactic acid content of other tissues is in equilibrium with that of the blood plasma, for lactic acid is freely diffusible across cell membranes (30) Under these circumstances the small amount of lactic acid which is present probably arises from a few special tissues, such as the red blood cells the intestinal mucous membrane, and the ret ina, etc Adult mammalian erythrocytes do not possess the enzymatic machinery for the use of oxygen but readily produce lactic acid from blood glucose (31, 32) The cells of the intestinal mucosa (33) and of the retina (34) have a high aerobic glycolysis (see p 55), that is they differ from most tissue cells, in which an ade quate oxygen supply inhibits lactic acid production (Pasteur effect [p 55])

In most tissues of the body lactic acid is not a necessary intermediate of carbo hydrate metabolism It is formed by the reduction of pyruvic acid only when the oxidative removal of the latter is relatively or absolutely deficient (p 49) A rela tive oxygen lack may occur during strenuous physical exercise, when the rate of oxygen supply to the muscles is temporarily inadequate in comparison with the rate of glycogenolysis (30), whereupon the lactic acid in the muscles increases and diffuses out into the blood Certain organs particularly the liver (35 36) but also the heart (37 38) will then remove the excess lactic acid from the blood and re oxidize it to pyruvic acid

An absolute lack of oxygen, leading to high lactic acid levels even when the body is at rest may result from pulmonary (39) or cardiovascular (40) diseases which interfere with the oxygenation of the blood or tissues, respectively A similar end result may be caused by liver disease (41), when the impairment of the oxida tive systems in this organ prevent it from utilizing the oxygen available in the blood for the removal and oxidation of the blood lactic acid

The importance of anoxia in lactic acid formation necessitates the same precau tions as for glycogen (p 78) when sampling tissues for chemical analysis The addition of sodium fluoride to blood prevents further glycolysis (42) Lactic acid is usually estimated by the method of Friedemann (43) or by that of Miller and Muntz (44) The latter method was modified and adapted to tissue analysis by Barker and Summerson (45)

PYRUVIC ACID

Since pyruvic acid is one of the most reactive metabolic intermediates (see p 52), it is not surprising that the amounts of pyruvic acid normally found in the blood and other tissues do not exceed 1 0 mg per cent (46, 47) The level rises

somewhat with the increased breakdown of carbohydrate accompanying muscular work (48) or following carbohydrate administration (46, 49) The pyruvic acid content of blood and tissues also increases during thiamine deficiency (50, 51), for many of the reactions which dispose of pyruvic acid require thiamine diphosphate as a coenzyme This fact has been used as an aid in the diagnosis of this avitaminosis (49, 51)

It should be noted that, despite the fact that pyruvic acid is by far one of the most important substances in intermediary metabolism, its normal concentration in blood and tissues is only about one tenth to one twentieth that of lactic acid This is because of the many mechanisms available for pyruvate removal (p 52), while lactic acid disposal is limited to one reaction—its oxidation to pyruvate This illustrates the general rule that the concentration of a substance in blood and tissues is not necessarily an indication of its importance in the metabolic scheme As we shall see presently, some metabolic intermediates are never present in detectable amounts unless special methods are employed to stop the metabolic reactions at that stage

The method commonly used for pyruvate estimation is that of Lu (52), or the subsequent modifications of this method (53, 54)

PHOSPHATE COMPOUNDS

We have already discussed the predominant role of compounds of phosphoric acid in carbohydrate assimilation and dissimilation (p 60) The phosphate derivatives group themselves into three classes inorganic phosphate, phosphorylated intermediates, and phosphate transfer substances

Inorganic phosphate (P_o) —The P_o in the body is largely derived from the inorganic phosphates present in foods Under certain circumstances the P_o of the blood may be increased by the mobilization of $Ca_3(PO_4)_2$ from the bones The P_o of blood and soft tissues may also rise as the result of an increased breakdown of organic phosphate compounds owing to anoxia or the interruption of the activity of certain enzyme systems Hence, the sampling of tissues for the correct estimation of P_o, as well as of the other phosphate derivatives, involves the same precautions as for glycogen (p 78) With more careful handling of tissues, lower P_o values have been reported (55) Table 8 summarizes the most reliable observations as to the levels of P_o and other important phosphate compounds in various bodily tissues

Phosphorylated intermediates —The only phosphorylated intermediates of carbohydrate metabolism which are normally present in the tissues in detectable quantities are (*a*) hexose-6 phosphate, (*b*) monophosphoglyceric acid, and (*c*) diphosphoglyceric acid (in red blood cells only) Table 8 lists the levels which have been reported The other known phosphorylated intermediates, such as glucose 1 phosphate, hexose diphosphate, etc , are metabolized as rapidly as they are pro-

duced and therefore are not found except when steps have been taken to interfere with their disposal (42, 56)

Phosphate transfer substances—This group consists of (*a*) adenosine diphosphate, (*b*) adenosine triphosphate, and (*c*) creatine phosphate The levels normally found in tissues appear in Table 8 The adenosine polyphosphates are present in

TABLE 8

DISTRIBUTION OF PHOSPHATE COMPOUNDS IN VARIOUS TISSUES OF MAN, RAT, RABBIT, AND DOG

Tissue	Inorganic Phosphate (P_i)	Creatine Phosphate (CrP)	Adenosine Di and Triphosphates (ADP and ATP)	Hexose-6-Phosphate (HMP)	Phosphoglycerate (PGly)	D phosphoglycerate (d PGly)	Total Acid-soluble Phosphate (P Total)	References
Skeletal muscle	15–25	50–70	30–40	8 15	40–50		150–200	(58, 62)
Cardiac muscle	23–29	5–13	18–28	14			80–100	(64)
Liver	18	0	15–25				90–100	(60)
Brain	7–9	9–11	16–19	4–6	0		70	(59 66)
Blood	3 5	0	10–20			30–50	50–80	(61)

TABLE 9

PROPERTIES OF THE VARIOUS ORGANIC PHOSPHATE COMPOUNDS (Robison and MacFarlane [71])

Procedure	Creatine Phosphate	ATP and ADP	Glucose-1 Phosphate	Glucose-6-Phosphate	Fructose-6-Phosphate	Hexose D phosphate	Triose Phosphate	Phosphoglycerate	Phosphopyruvate
I Percentage of hydrolysis in molybdate at 25° C for 30 minutes	100	0	0	0	0	0	0	0	0
II Percentage of hydrolysis in *N* HCl at 100° C									
In 7 minutes		100	100			32	46		
In 30 minutes				2	24	59	92	1	93
In 60 minutes				3	45	72	100	2	100
In 180 minutes				9	84	94		6	
III Percentage of hydrolysis in *N* NaOH at 20° C in 15 minutes						0	100		0
IV Reducing power per 100 mg of the free ester, compared to glucose as 100			0	55	55	21	50	0	

all tissues of the body to a greater or lesser extent (57, 58 59 60 61) However creatine phosphate seems to be limited to *contractile and conducting tissues*—i e , striated smooth, and cardiac muscle, neurones, and nerve fibers (62 63 64 65 66) It has also been found in spermatozoa (67) There is no creatine phosphate in blood or liver (60 61)

Analytical methods —The methods for the estimation of the various phosphate compounds are based upon separation of the desired compounds from each other by the differential solubility of their barium salts (68, 69) and the varying conditions under which the inorganic phosphate portion can be split off the particular organic substances (70 71, 72) Table 9 outlines the principles underlying the various determinations A reliable method for the estimation of P_o must be used of course, in all such procedures (73 74)

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CHAPTER VII

SITE OF ORIGIN OF BLOOD SUGAR

IT IS well established that in the fasting animal the liver is virtually the sole source of the blood sugar (1 2 3) There is some recent evidence that the kidney may contribute sugar to the blood but in amounts that are hardly ignificant in relation to the total carbohydrate requirements of the normal intact nimal (4 5) The other tissues of the body continually require and use the blood ugar for the maintenance of their metabolism and functions Since the blood ugar level is well maintained throughout long periods of fasting it is evident that he sugar which the liver secretes into the blood under these conditions must be lerived from stored carbohydrate or non carbohydrate precursors It has been riefly indicated in the previous chapters that the storage form of carbohydrate is lycogen and that the non carbohydrate precursors are protein and fat The present and following chapters will consider the evidence for these interconversions in ome detail

The brilliant pioneer work of Claude Bernard was the first to indicate the predominant role of the liver in supplying blood sugar and to demonstrate the existence of liver glycogen His early reports claimed that in fasting animals or those fed on meat the blood entering the liver through the portal vein contained no sugar (6) Repetition of these experiments by some of his contemporaries led to disagreement and controversy for they found sugar in the portal vein blood As it turned out the reasons for these differences lay in the then inadequate knowledge concerning the proper handling of blood samples and the crude methods for sugar analysis Bernard and his contemporaries eventually agreed that while sugar was constantly present in the portal blood there was always more sugar in the blood leaving the liver (7)

Claude Bernard also demonstrated that a liver flushed free of sugar by perfusion with cold water acquired a high sugar content after a few hours in the laboratory He recognized the starchlike nature of the precursor of this sugar and called it glycogen He confirmed Chauveau in the finding that the sugar of arterial blood throughout the body was higher than that of venous blood On the basis of these essential facts and a number of other observations Bernard arrived at the following conception which is as valid today as when he enunciated it

In the liver sugar is produced although a little is also destroyed in that organ in the muscles sugar is destroyed Destruction of sugar probably occurs throughout the organism in all the

THE LACTIC ACID CYCLE

The evidence which has been cited also shows that, once sugar has entered the peripheral tissues, even though it is stored rather than used, it cannot re ente the blood as glucose This, of course, is in accord with what is known of the en zyme systems in skeletal muscle (p 34) However, under special circumstances significant amounts of carbohydrate can leave the muscle in altered form, as when lactic acid accumulates in the muscle and diffuses into the blood stream This oc curs during a relative or absolute deficiency in the oxygen supply to the muscle (see p 49) At such times the lactic acid may be carried to the liver and converted into hepatic glycogen, and thus eventually reappear as blood sugar This so-called "lactic acid cycle" has been investigated and elaborated by Geiger (25, 26, 27), Himwich (28), Cori (29), and others But it is fair to say that, while it constitutes a possible indirect source for some blood sugar during abnormal or emergency con ditions, it is of little or no significance as regards the blood sugar supply under normal conditions

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An entering wedge into the solution of the problem was made by Hershey and Soskin (42, 43), who showed that it was not the digestive-enzyme activity of the administered pancreas that was essential for the relief of the fatty liver and the accompanying syndrome of "liver failure," as had previously been supposed They demonstrated the same effects by feeding a preparation of egg yolk lecithin Further work by Best, Hershey, and Huntsman (44) revealed that it was the choline constituent of the lecithin molecule that exerted all the physiological activity Since then, the literature on choline and other substances with similar activity ("lipotropic" factors) has grown enormously (45), and a complete review of this subject would take us far afield What is pertinent to the present discussion is the observation of Ralli *et al* (46) that the lipotropic activity of raw pancreas was greater than could be accounted for by its lecithin or choline content

In 1936 Dragstedt and his associates (47, 48) began an important series of investigations by preparing an active pancreatic extract which, despite its low choline content, was a very effective lipotropic agent in the depancreatized dog They named the active principle "lipocaic" and tentatively considered it to be a hormone, because occlusion of the external pancreatic ducts of normal dogs did not result in any evidences of the lack of the lipotropic substance The hormonal nature of lipocaic has been challenged by the laboratories of Chaikoff (49, 50, 51) and of Ralli (46, 52), which have reported (*a*) that, in their hands, ligation of the pancreatic ducts does produce a fatty liver and (*b*) that the oral administration of the external secretion of the pancreas (pancreatic juice) yields as great a lipotropic effect as the feeding of raw pancreas These contradictory results and conclusions have not yet been resolved What concerns us for the moment, however, is the area of agreement (53, 54), i e , that the pancreas secretes, whether internally or externally, a lipotropic agent other than, or in addition to, choline

The subject has been complicated by the use, by various investigators, of animals other than the dog and methods other than pancreatectomy In a comprehensive review of the literature on lipotropic factors McHenry and Patterson (45) reached conclusions which may be summarized as follows

1 There are different kinds of fatty livers, depending upon how they are produced and differing in the chemical composition of the liver lipids (see Table 10)

2 When the fatty liver contains a high percentage of neutral fat, choline is an effective lipotropic agent

3 When the fatty liver contains a considerable percentage of cholesterol, lipocaic and inositol are more effective agents than choline

nicious anemia factor

Wherever the future work on lipocaic and other lipotropic substances may lead, it is clear that, in dealing with the depancreatized dog, one must provide *lipo-*

tropic agents adequate in kind and amount to prevent fatty infiltration and preserve the functional integrity of the liver

PHLORHIZIN DIABETES

So-called 'phlorhizin diabetes" was discovered and first described by von Mering in 1879 (12) It results from the administration to experimental animals of the

TABLE 10*

COMPARISON OF THE EFFECTS OF LIPOTROPIC FACTORS
(MCHENRY AND PATTERSON [45])

Regimen Used for Production of Fatty Livers	Choline	Lipocaic	Inositol
Depancreatized dogs	++*	++*	−?
Rats			
High fat diet			
Thiamine	++*	o	−
All B vitamins	++*	−	−
Cholesterol	+*	o	+
Fat free diet			
Th amine	++*	−	o
Thiam ne and riboflavin	++*	−	−
Thiamine riboflavin pyridoxine and pantothenic acid	+*	−	+*
Above four vitamins and b otin	o	++	++
B vitamins and cholesterol	+	+	+

*Symbols ++ strong l potropic action + mode ate lipotropic action o no lipotropic action − lack of data * verified in two or more laboratories

glucoside phlorhizin (or phlorhidzin), which has the structure indicated in Figure 27 The drug is generally administered subcutaneously as a fine suspension in oil, and the usual dosage is about 1 gm of phlorhizin per day for a 10-kg dog (13)

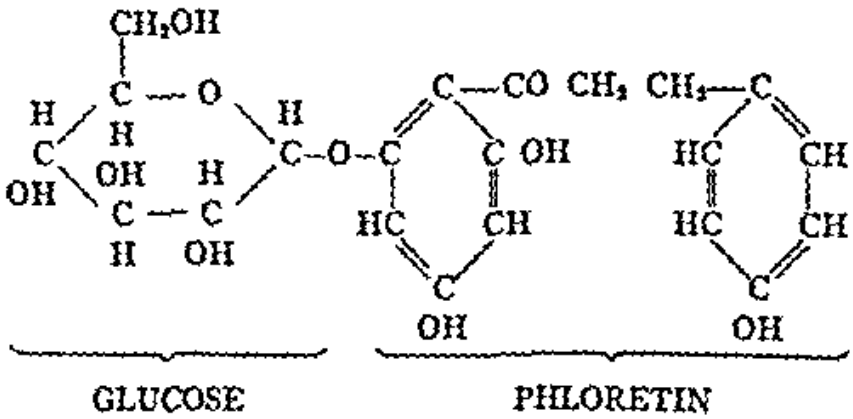


FIG 27.—Phlorhizin

In order to obtain a rapid initial effect the first dose is sometimes administered in a 2 5 per cent sodium bicarbonate solution (14)

The syndrome of phlorhizin diabetes (15) and its progression to the death of the animal resembles that of pancreatic diabetes in practically every particular ex

cept that the blood sugar level is abnormally low (hypoglycemia), as opposed to the hyperglycemia of the depancreatized animal As has been previously indicated (p 77), the drug produces its effect by preventing the reabsorption of sugar by the tubules of the kidney This is accomplished by the inhibition of the phosphorylation of the glucose to hexosephosphate (16) All tissues are subject to the same action of phlorhizin But muscle tissue destroys phlorhizin very quickly, so that effective concentrations of the drug in muscle are not attained by the procedure employed in producing phlorhizin diabetes in the living animal (17, 18) However, under *in vitro* conditions the action of phlorhizin on isolated muscle tissue can be readily demonstrated (19) As used *in vivo*, the kidney shows the greatest effects of phlorhizin because it has a limited ability to destroy the drug (17) and also because the excretory function of the kidney leads to the accumulation of phlorhizin in larger concentration than elsewhere in the body (15) Hence, phlorhizin diabetes may be regarded as being primarily a disturbance in the kidney This was shown at an early date by Minkowski, who demonstrated that the removal of the kidneys from phlorhizinized dogs abolished all signs and symptoms of diabetes during the time of survival of the animals in the absence of renal excretory function (11)

A comparison of pancreatic and of phlorhizin diabetes indicates that the polyuria, polydipsia, dehydration and demineralization, loss of weight, weakness and polyphagia, and ketosis and coma are dependent, in both, on the loss of significant quantities of carbohydrate from the body by way of the urine In pancreatic diabetes, this results from a disturbance in the regulation of the blood sugar, leading to hyperglycemia, which, in turn, exceeds the capacity of the phosphorylative mechanism of the kidney for the reabsorption of sugar In phlorhizin diabetes, the same train of events is initiated by a lowering of the phosphorylative capacity of the kidney, allowing a significant excretion of sugar at normal and hypoglycemic blood sugar levels

THE NON UTILIZATION THEORY OF DIABETES

During the ten years that followed the discovery of pancreatic diabetes by von Mering and Minkowski in Strassburg, the same laboratory established the classical criteria of the metabolic disturbance in experimental diabetes (20) These criteria comprise (1) the quantitative excretion of administered carbohydrate in the urine of the experimental animal, (2) the urinary dextrose to nitrogen ratio (D N), (3) the excretion in the urine of acetoacetic acid, β hydroxybutyric acid, and acetone (ketosis), and (4) the characteristic respiratory quotient (R Q)

The quantitative excretion of administered sugar by the diabetic animal suggested that the cause of the metabolic difficulty was an inability to utilize carbohydrate (the non utilization theory) Furthermore, when Minkowski collected urine specimens from his depancreatized dogs (while fasting or when fed lean

meat) and analyzed them for amounts of dextrose and nitrogen, respectively, the total amount of sugar in each 24 hour specimen seemed to bear a definite relationship to the amount of nitrogen in the same specimen (6, 11) This D N ratio aver aged about 2 8 1 for his animals (see Table 11), from which he concluded as fol

TABLE 11

ORIGINAL DATA OF MINKOWSKI (11) ON SUGAR AND NITROGEN EXCRETION OF DEPANCREATIZED DOGS

Dog No	Weight (Kg)	Duration of Diabetes (Days)	Duration of Meat feeding (Days)	Amount of Meat (Gm per Day)	Urine Sugar (Gm per 24 Hr)	Urine Nitrogen (Gm per 24 Hr)	D N Ratio
1	15	2	1	500	102 0	32 2	3 14
		3	2	750	61 1	21 2	2 88
		4	3	750	89 1	30 3	2 94
		5	4	500	44 7	14 4	3 09
		9	2	750	69 0	24 4	2 96
		10	3	750	(5 6%)	(1 98%)	2 83
2	13	12		650	54 0	17 45	3 09
		13	2	650	61 4	21 12	2 91
3	12	9	3				
		10	7	500	34 8	12 76	2 72
		11	8	500	42 0	14 05	2 99
		13	9	500	61 2	20 19	3 06
		15	11	500	60 8	20 37	2 90
4	12	8	13	500	40 0	13 73	2 88
		9	3	1 000	48 4	17 6	2 74
		10	4	1 000	62 6	21 9	2 86
5	9	3	5	1 000	53 6	17 5	3 05
		4	2	500	43 2	14 26	3 03
6	9	7	3	500	37 0	12 45	2 97
	8	2	?		(4 9%)	(1 6%)	3 05
7		6		300	20 2	6 4	3 16
		8	1	300	19 1	6 3	3 03
8	6	11	3	300	20 2	6 7	2 93
		5	2	500	27 3	10 12	2 70
		6	2	500	24 4	8 73	2 72
9	5	7	3	500	34 3	11 90	2 88
		8	4	500	30 0	11 10	2 70
		11	5	300	12 8	4 88	2 62
		12	2	300	14 5	5 45	2 66
		13	3	300	15 1	5 46	2 76
		14	4	300	16 0	5 95	2 69
		15	5	300	12 4	4 20	2 95
			6				

lows (*a*) Since nitrogen is a breakdown product of protein, all the sugar which ap peared in the urine was being made at the expense of protein (*b*) From the apparent constancy of the D N ratio, none of the sugar made from protein was being utilized by the diabetic animal, i e , all of it was quantitatively excreted

The appearance of the ketone bodies in the diabetic animal was the third basis for the non utilization theory of diabetes It was known that acetoacetic acid and β hydroxybutyric acid resulted chiefly from the breakdown of fat Since these substances did not ordinarily appear during fasting in the normal organism (when fat was the chief metabolite), it was assumed that the ketone bodies were abnormal waste products resulting from the incomplete oxidation of fats in diabetes From this arose the conception that a certain amount of carbohydrate had to be oxidized in order that fats could be burned completely ("fats burn in the fire of carbohydrates") (21, 22, 23) Thus the ketosis of diabetes was apparently another evidence of the lack of ability to utilize carbohydrate

Studies of the respiratory exchange of the normal and diabetic organism apparently supported the foregoing conclusions If the net result of complete oxidation in the body is compared to the burning of a substance in a bomb calorimeter, it is apparent that the amount of oxygen consumed and the amount of CO_2 given off in the process will depend upon the chemical nature of the substance that is being oxidized Thus it may be calculated that, when a carbohydrate is oxidized, 1 mol of CO_2 will result for every mol of oxygen used, according to the reaction

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

The R Q is the relation expressed in volumes, between the oxygen consumed and the CO_2 given off (CO_2/O_2) Hence the R Q for the oxidation of carbohydrate is 1 0 In the same way, it may be calculated that the R Q for fat is about 0 7, for protein, about 0 8 The latter figure involves a number of assumptions, since protein is not entirely oxidized in the living organism (24, 25)

It was found that the R Q of a normal animal under fasting conditions was in the neighborhood of 0 7 This was taken to indicate that fat was the chief fuel being used at that time After a carbohydrate meal the R Q of the normal animal rose toward 1 0 (Fig 28) This was interpreted to mean that the animal was now oxidizing the ingested carbohydrate The diabetic organism differed from the normal in that, while its fasting R Q was also about 0 7, the quotient did not rise when carbohydrate was administered (Fig 28) This seemed to confirm the conclusion that the diabetic organism cannot use carbohydrate but derives its energy chiefly from fat (24, 26, 27)

A CRUCIAL EXPERIMENT OPPOSING THE NON UTILIZATION THEORY OF DIABETES

On the basis of the four lines of evidence which have been outlined, the non utilization theory of diabetes was more or less generally accepted for many years This was made possible by ignoring certain inconsistencies in the evidence and by neglecting other evidence to the contrary As early as 1897, Kausch (28) reported the results of removal of the liver from depancreatized geese and ducks, as com

pared to the results of the same procedure in normal birds He found that, in the absence of the organ which supplies the blood sugar, the latter disappeared from the blood just as quickly in the diabetic birds as in the normal ones There were a number of subsequent attempts to confirm this finding in mammals Most of them showed similar results (29 30, 31), but technical difficulties as regards complete removal of the liver and the consequent irregularity of the data rendered these findings inconclusive

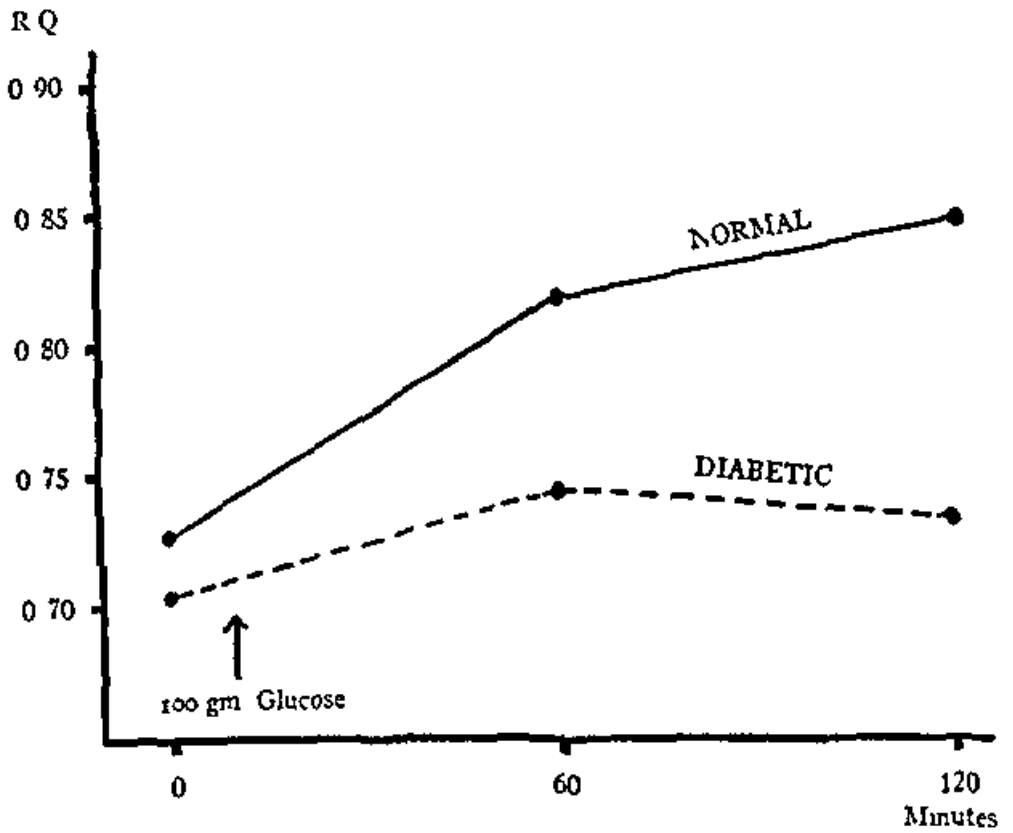


FIG 28 —Rise of R Q following sugar administration to normal and depancreatized dogs (From the data of Barker *et al* [26])

However, following the development of Mann s technic for total removal of the liver in dogs Mann and Magath (32) reported unequivocal evidence that the completely depancreatized dog suffers just as rapid a fall in the blood sugar after hepatectomy as does the normal dog (Fig 29) Whether originally normal or diabetic, the liverless animal dies in hypoglycemic convulsions within a few hours In either case it can be kept alive only by continuous administration of sugar or the giving of larger amounts of sugar at about 2 hour intervals Unless one makes the rather absurd assumption that the removal of the liver suddenly restores the ability of the peripheral tissues to utilize carbohydrate, one must conclude that the diabetic animal does not lack that ability Under these circumstances it be-

comes important to re examine the classical criteria of diabetes for their true mean ing and to consider all other evidence which may help to explain the diabetic syn drome without invoking the non utilization theory

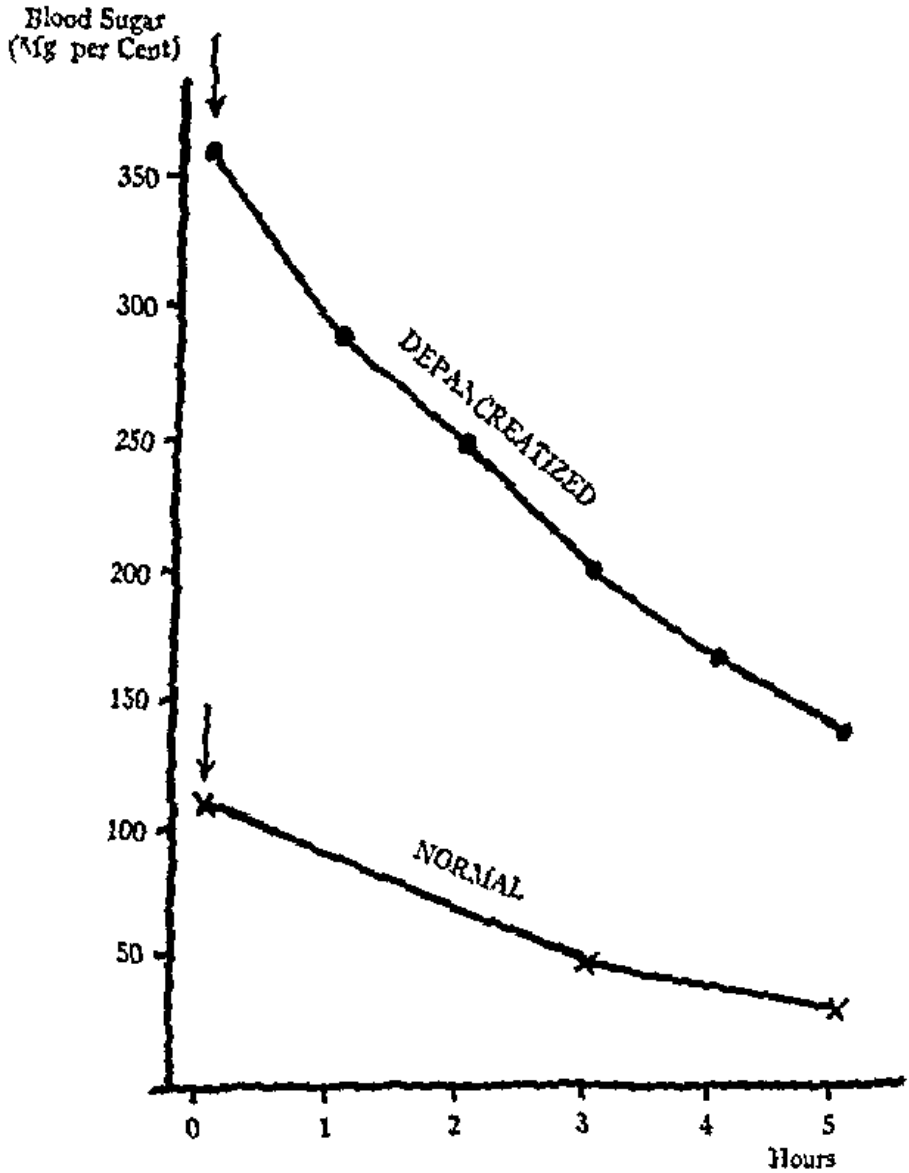
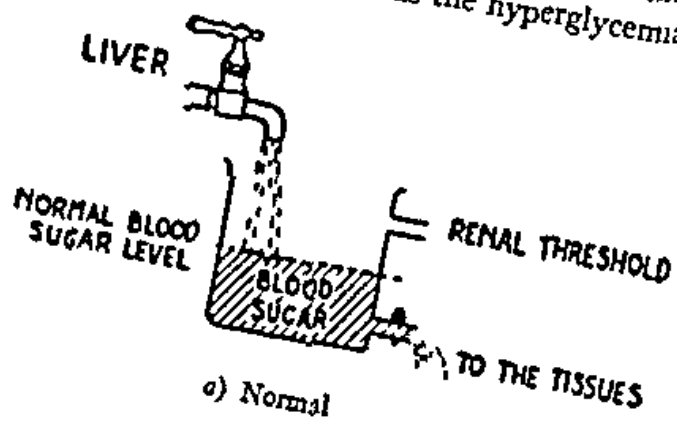


FIG 29 —Development of hypoglycemia follow ing hepatectomy in depancreatized as well as in nor mal dogs (Mann and Magath [32])

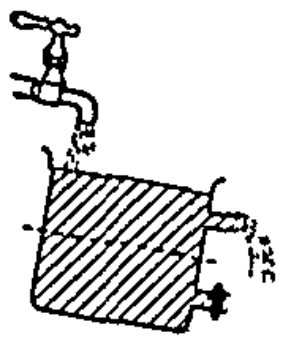
THE OVERPRODUCTION THEORY OF DIABETES

The alternative to the non utilization theory of diabetes is the overproduction theory of diabetes These two possible explanations for the diabetic syndrome are compared in Figure 30 in terms of a simple mechanical analogy Diagram *A* indi cates the state of affairs in a normal animal in which the liver, as represented by

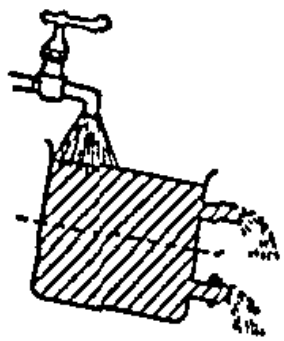
the tap, is pouring just as much sugar into the blood as the tissues (represented by the lower, outflow tube) are drawing off for utilization The net result of the dy namic balance between inflow and outflow is the normal blood sugar level Dia gram *B* represents the non utilization theory adopted by Minkowski Here the outflow of sugar into the tissues has ceased while the liver continues to pour sugar into the blood The blood sugar rises and as the hyperglycemia approaches the



a) Normal



b) Non utilization



c) Overproduction

Diabetic

FIG 30.—Mechanical analogy illustrating the alternative theories of diabetes

renal threshold glycosuria begins Diagram *C* represents the other possible ex planation first proposed by von Noorden (*33*) and later advocated by a vigorous minority (*34 35 36*), namely, the overproduction theory Here, there is no dimi nution of the utilization of blood sugar by the tissues But the supply of sugar to the blood from the liver has become excessive to the point where continued normal utilization can no longer keep pace with it Hyperglycemia and glycosuria follow

Figure 30 makes it obvious that closing the tap (hepatectomy) would produce the same end result, namely, emptying of the tank (hypoglycemia) in diagrams *A*

and *C* but not in diagram *B* Thus, while both theories can account for cardinal features of the diabetic syndrome, the non utilization theory is directly opposed by the hypoglycemic effect of hepatectomy in the diabetic animal (p 97) There is no conflict in this regard if one adopts the overproduction theory The re examination of the classical criteria of diabetes which is the subject matter of the subsequent three chapters should, therefore, be followed with reference to both the possibilities indicated in Figure 30

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PART III

CRITICAL SURVEY OF THE CLASSICAL CRITERIA OF DIABETES

CHAPTER IX

QUANTITATIVE EXCRETION OF ADMINISTERED SUGAR AND THE DEXTROSE NITROGEN RATIO

THE fact that the administration of dextrose to his diabetic animals resulted in the excretion of a roughly equivalent amount of sugar in the urine led Minkowski to advocate the non utilization theory Reference to Figure 30 (p 99) will show at a glance that his conclusion was not a logical necessity It may be seen that viewed from the standpoint of either theory the influx of an extra amount of sugar into the blood would be expected to result in an extra outflow of the same amount of sugar in addition to that which is already overflowing through the kidneys

DEXTROSE NITROGEN RATIO IN THE DEPANCREATIZED ANIMAL

It is important to consider in detail the supposed constancy of the D N ratio If it were truly constant it would constitute strong support for the non utilization theory For it would be difficult to conceive of a rate of sugar utilization (other than zero utilization) so unvarying in different diabetic animals and under different conditions as to make the ratio possible Minkowski s summarized data are reproduced in Table 11 (p 95) On 31 experimental days in 9 depancreatized dogs fed on meat he obtained D N ratios which varied from 3 16 1 to 2 62 1 with an average of 2 8 1 (1) The experimental days which he used to establish the average ratio were admittedly selected since a record of all the experimental days on any single animal would show D N ratios much higher than 2 8 1 to begin with and also ratios which fell progressively below this figure as the exitus of the animal was approached The high initial D N ratios were discarded on the basis that they represented the pouring out of preformed glycogen stores The low D N ratios toward the end of the experiments were disregarded because of the poor condition of the animals at that time The reasonableness of these objections to the results selected in a much more arbitrary manner than we have been led to believe by those who have trustingly accepted his average as a physiological constant The examination of Minkowski s data makes it apparent that the experimental days were of the first and last few days of each experiment cannot be denied But a closer analysis in Table 12 shows that the selected data in any given experimental animal began as early as the second day of diabetes or as late as the twelfth day and ended as early as the fourth day of diabetes or as late as the fifteenth day

Moreover, the days reported in some experiments are not consecutive, some days being omitted, for no stated reason It must be apparent that any desired average D N ratio might have been obtained by such arbitrary selection of experimental days, picked from experiments in which the D N ratios fell progressively from high to low values

This criticism is supported by other results of Minkowski—reported in the same paper but not included in the figures from which he obtained his D N ratio In comparing the initial D N ratios obtained from well nourished and poorly nourished animals he recorded ratios in the latter animals of 2 04, 2 43, 1 62, and 2 24 on the third, fourth, and fifth days of diabetes It is difficult to understand why Minkowski did not attempt to correlate these low results with the data from

TABLE 12

THE DAYS DURING THE DIABETIC LIFE OF HIS DOGS WHICH MINKOWSKI (1) USED TO COMPUTE HIS AVERAGE D N RATIO

Dog No	Days after Pancreatectomy														
I		2	3	4	5				9	10					
II												12	13		
III									9	10	11		13		15
IV								8	9	10					
V			3	4											
VI							7								
VII						6		8			11				
VIII					5	6	7	8							
IX											11	12	13	14	15

which he computed his average ratio The poor nutrition of these animals might perhaps have accounted for the failure to obtain high initial D N ratios But the *values uniformly below 2 8 1 obtained on days in which the approaching* demise of the animal was not a factor and on days which coincided, in point of time, with some of the experimental days which were used to obtain his average, serve to confirm the arbitrary nature of the average D N ratio at which he arrived This indication of the inherent defects in Minkowski s work is not intended to cast aspersions on his integrity as a physiologist It must be remembered that Minkowski, working before the days of insulin, had to deal with acutely diabetic dogs suffering from the effects of a recent anesthetic and operation

Pflueger (2), Embden (3), and others subsequently reported that they had failed to obtain fixed D N ratios at the Minkowski level Their work was criticized on the assumed ground of the poor condition of their animals or of incomplete pancreatectomy Such criticism, however, cannot be leveled at the work of Macleod and Markowitz (4), who used depancreatized dogs that were maintained in excellent condition by the use of insulin After the withdrawal of food and insulin from such animals (which by subsequent post mortem examination were shown to

be completely depancreatized) they obtained D N ratios far below 2 8 1, after the first few days of the experiment had elapsed Chaikoff and co-workers (5) reported similar results and found (as noted by Minkowski) that the D N ratio was generally higher in fat than in lean dogs and also that it varied decidedly in the same animal according to its nutritional condition at the time of the experiment

In 1930 Rapport (6) reviewed the extensive literature on the D N ratio in addition to the above and was not able to reconcile the large variations which had been reported In the same year Soskin (7) published a comprehensive reinvestigation of the D N ratio in depancreatized dogs using the advanced technique made possible by the discovery of insulin This work was done on depancreatized dogs which were completely recovered from operation by the use of insulin and present

TABLE 13

DISTRIBUTION OF D N RATIOS DURING 138 UNSELECTED DAYS FOR 10 DEPANCREATIZED DOGS (SOSKIN [7])

Range of D N ratio	No of Experimental Days on Which Results Were Obtained (Total 138 Days)				
	5	33	43	36	1
	Over 3 16	3 16–2 63*	2 6 2 00	1 99–1 00	Less than 1 00

*Minkowski's range

ed well healed and non infected wounds The animals were maintained on a low caloric protein diet and the absence of islet tissue was verified by post mortem examination In contrast to Minkowski s animals they survived the withdrawal of insulin for as long as 5 weeks during which time they usually remained bright and active although losing weight The observations on the D N ratio comprised 138 unselected days for 10 dogs in contrast to Minkowski s 31 selected days for 9 dogs The distribution of the D N values obtained is shown in Table 13 It may be seen that although some D N ratios similar to Minkowski s were obtained there is nothing to indicate that such values have any particular significance In general the D N ratio tended to be high at the beginning of each experiment and to show a progressive fall as the animals lost weight and their stores of adipose tissue were depleted This serves to explain the different D N ratios reported by previous workers The fact that some animals maintained D N values far below 2 0 1 for as long as 18 days precludes the appellation of premortal which some writers have used to avoid consideration of all ratios below the Minkowski level (8) It is clear that if Minkowski s interpretation of his ratio is accepted the progressively lower ratios obtained later in the experiments signify the utilization of increasing amounts of the sugar arising from protein If on the other hand the

low ratios obtained later in the experiments represent the true extent of gluconeogenesis from protein, the higher Minkowski values must mean that sugar is being formed from fatty acid as well as from protein In either case, there remains no basis for concluding that sugar is derived solely from protein or that none of the sugar so formed is utilized by the diabetic organism It is permissible to conclude that sugar is derived partly from protein, but it is impossible to say to what extent this occurs

DEXTROSE NITROGEN RATIO IN THE PHLORHIZINIZED ANIMAL

Conclusions similar to those arrived at with respect to pancreatic diabetes may be drawn in regard to the significance of the D N ratio of 3 65 1 obtained by some investigators in so called "phlorhizin diabetes" There is an added difficulty in interpreting this type of work in that there is no standard for judging the experimental preparation, comparable to the histological demonstration of complete pancreatectomy in operated animals It is obviously fallacious to account for different D N ratios obtained with phlorhizin in different animals and by different workers (15) by saying that some of the animals were not completely phlorhizinized because they did not yield D N ratios of 3 65 1 An added complication is the fact that the phlorhizin, as used, is not a pure chemical substance of known composition In his last publication on the subject, Graham Lusk (9) (who together with his co workers had made the most extensive use of phlorhizin diabetes in their studies) confessed that with the phlorhizin he was then able to obtain he could not reproduce the D N ratio of 3 65 1 which he had formerly insisted was the necessary criterion for complete phlorhizinization

Even those workers who used preparations of phlorhizin with which they were able to obtain some D N ratios approximating 3 65 1 were not able to maintain such ratios at will in a given animal As in the depancreatized organism, the D N ratio resulting from continued phlorhizin administration starts at a high value and declines progressively The selection of days upon which the ratio is to be considered a valid one is a purely arbitrary matter Table 14 and Figure 31 show the day by day excretion of sugar and nitrogen in the urine and the D N ratio in three dogs receiving the customary phlorhizin treatment It may be seen that there is no evidence for a constant D N ratio at any level

If, for the moment, one were to discount the foregoing considerations, one would still have to explain the difference between the phlorhizin D N ratio of 3 65 1 and the Minkowski ratio of 2 8 1 There is no factual basis for concluding that phlorhizin alters the biochemical processes in such a manner as to allow a larger proportion of the protein molecule to be converted into sugar And, if a constant proportion of the protein molecule is convertible, one or both of the following conclusions is justified either the depancreatized animal always utilizes a significant fraction

of the sugar derived from protein or the phlorhizinized animal must be forming sugar from fat as well as from protein

Finally, one must take into account the fact that even the classical criteria are self contradictory as regards the ability of phlorhizinized animals to utilize carbohydrate

a) Insulin has been obtained from the pancreas of dogs after prolonged and maximal phlorhizin treatment (10)

TABLE 14

LACK OF CONSTANT D N RATIO IN FASTED PHLORHIZINIZED DOGS

Dog No	Length of Expt (Days)	Urine Excretion (Gm per Day)		D N	Urine Ketones	Blood Sugar (Mg per Cent)
		Dextrose	N troge n			
1	1	16 05	5 95	2 69	++	14
	2	11 16	4 43	2 52	+++	21
	3	9 36	3 70	2 53	+++	18
	4	6 22	2 89	2 15	++	26
	5	2 26	1 73	1 31	++	
	6	3 80	2 83	1 35	++	14
	7	4 06	2 84	1 43	+++	12
	8	2 85	2 72	1 05	0	31
2	1	6 46	2 31	2 79	0	38
	2	5 55	3 11	1 79	0	33
	3	3 69	1 94	1 90	0	33
	4	4 09	2 36	1 73	0	20
	5	4 89	2 36	2 07	+	11
	6	2 51	1 86	1 35	++	
	7	2 57	1 56	1 65	0	17
	8	1 87	1 40	1 26	0	31
	9	3 15	2 09	1 50	0	13
3	1	24 61	7 84	3 14	++	30
	2	15 86	6 58	2 41	+	29
	3	13 18	5 86	2 25	+++	24
	4	11 00	6 41	1 71	+++	28
	5	9 89	5 23	1 89	++	22
	6	8 11	4 77	1 70	+	18
	7	9 53	4 52	2 11	0	
	8	8 13	4 40	1 85	+	21
	9	7 92	4 61	1 72	++	15
	10	5 49	3 82	1 44	0	14
	11	5 14	4 26	1 23	0	20

b) After nephrectomy the phlorhizinized dog is quite normal as regards its blood sugar level its R Q and the rise in the R Q following glucose administration (11, 12)

c) The ingestion of sugar by the intact phlorhizinized animal results in the retention of glucose which has an antiketogenic and protein sparing action, and causes a rise in the R Q comparable to that which occurs in the normal dog (11, 13, 14, 15 16)

CHAPTER X

KETOSIS

OF THE three substances usually grouped under the term ' ketone bodies namely, acetoacetic acid, β hydroxybutyric acid, and acetone the second is *not* a ketone, and the third represents merely a breakdown product of its more physiologically significant precursors It is now generally agreed that, under conditions leading to ketosis, acetoacetic acid is the first ketone body to be formed (1) It is known that various tissues of the mammalian organism are able to reduce acetoacetic acid to β hydroxybutryic acid and also to effect the reverse reaction The direction of this reversible reaction depends on the concentration of substrates present and on the oxygen tension, and there is evidence that an equilibrium between these two substances is established rapidly (2, 3 4) Hence it is a matter of practical importance, in balance or recovery experiments, to estimate the amounts of both of these substances present in the tissues when attempting to account for the fate of a given amount of either Acetone is readily formed in solutions containing acetoacetic acid, and it is generally assumed that whenever it is found in biologic fluids it is merely a spontaneous decomposition product which indicates that an equivalent amount of one of the other ketone bodies was formerly present

SITE OF ORIGIN OF THE KETONE BODIES

Practically all investigators have agreed as to the chief source of the ketone

bodies A similar conclusion regarding *ketogenesis by these organs in situ* was reached by Himwich, Goldfarb and Weller (9 10), who compared the ketone levels of the inflowing arterial blood and of the outflowing venous blood of the vari

blood leav

the reverse

as an occa

sional output of small amounts of ketone bodies from the skeletal muscles and the intestinal tract In agreement with this, Jowett and Quastel (11) found that slices of kidney, spleen, testis, and brain *in vitro* could produce small amounts of the ketone bodies from butyric acid but that liver slices under similar conditions produced from ten to forty times as much

It should be noted that the evidence quoted above does not prove that organs other than the liver are incapable of forming considerable amounts of the ketone bodies For it is obvious that when a tissue is capable of utilizing a substance, the amount of the latter which may escape from that tissue into the blood (or surrounding medium *in vitro*) is merely the difference between the amount formed and the amount utilized *in situ* That this is not a theoretical consideration only was shown by Weinhouse (63) for kidney tissue, using the heavy carbon tracer technic Under these circumstances the role of the liver as the chief site of origin of ketone bodies depends upon the fact that it can form these substances at a much greater rate than it can utilize them

Whether or not the extrahepatic tissues can be shown to put out some ketone bodies under special experimental conditions, it is clear that in the living intact animal the liver is practically the sole source for these substances Thus it has been demonstrated that dogs in which the functional capacity of the liver is limited by an Eck fistula do not exhibit increased ketosis after phlorhizin administration (12) The reduction of hepatic function by hepatotoxic agents also decreases

the diabetic animals was due to rapid ketogenesis in the liver Finally Mirsky (15) has recently shown that the ketogenic effects of certain pituitary extracts which are regularly obtained in normal animals cannot be demonstrated in the absence of the liver

SOURCE MATERIALS FOR PRODUCTION OF KETONE BODIES

The early work of Embden and co-workers (16 17) indicated the formation of extra ketones by perfused livers when fatty acids certain amino acids or pyruvic acid were added to the perfusing fluid These three different source materials for the ketone bodies have since been confirmed by a number of investigators in a variety of ways (18 19 20 21 22 23) However, Embden and associates reported that the amount of ketone bodies arising from fat greatly exceeded that from the other sources Subsequent work has emphasized the fact that, when ketosis occurs in the living organism it may be regarded for practical purposes as an index of the catabolism of fat Thus the perfused fatty liver produces much greater amounts of the ketone bodies than the liver that is poor in fat (23) The livers of depancreatized or phlorhizinized animals which are characteristically rich in fat, are known to produce excessive amounts of ketone bodies (22) In the intact normal animal the feeding of fat or the excessive use of depot fat, induced by starvation, results in ketosis More recently Stadie Zapp, and Lukens (24 25) have demonstrated that the production of ketones by liver slices *in vitro* is accompanied by the disappearance of amounts of fatty acid sufficient to account for more than 1 mol of ketone per molecule of fatty acid

MECHANISM OF PRODUCTION OF KETONE BODIES FROM FATTY ACIDS

For many years the general conception of the mechanism by which ketones are formed from fatty acids seemed to be quite settled, but it has recently undergone at least two metamorphoses The theory of successive β oxidation originated from the work of Knoop (26) It was based on the feeding of various phenyl substituted fatty acids to test animals and the identification of the excretion products in the urine The administration of either benzoic, phenylpropionic, or phenylvaleric acid resulted in the appearance of hippuric acid After the administration of phenyl acetic and phenylbutyric acids, phenylaceturic acid appeared in the urine (Fig 32) These results could be reasonably explained only by assuming that the fatty acids were degraded by the splitting off of two carbon atoms at a time, by oxida tion at the carbon atom which occupied the β position in relation to the carboxyl group It was assumed that the acetic acid molecules so formed were rapidly metab olized, while the phenyl group was left attached to one or two carbon atoms, de pending on the original number of carbon atoms in the fatty acid molecule This assumption was confirmed *in vivo* by Dakin and was extended to the *in vitro* oxida tion of various fatty acids by hydrogen peroxide at body temperature (27, 28 29) Snapper, Gruenbaum, and Neuberg (7) duplicated Knoop s results on the per fused kidney

With this groundwork laid Embden and co workers (5, 6) perfused various fatty acids through isolated livers and reported that ketones were formed from fatty acids with an even number of carbon atoms in the molecule but not from the odd numbered fatty acids This confirmed the natural occurrence of β oxidation and also seemed to indicate that the last four carbon atoms in the chain under went oxidation at the β position but were not split It was, therefore, assumed that each molecule of an even numbered fatty acid, regardless of chain length, resulted in the production of one molecule of ketone and that odd numbered fatty acid could not give rise to ketone bodies On this basis, also, the amount of oxygen re quired for the degradation of a given fatty acid and the production of one molecule of ketone could be calculated (Fig 33)

Although this conception gained wide popularity (especially among clinicians concerned with clinical states characterized by ketosis) and although it persists in many textbooks up to the present day, serious objections from the experimental standpoint arose before many years had passed Thus, Hurtley (30) sought for the butyric and acetic acids that would be expected to be present in the liver during active ketogenesis and failed to find them Clutterbuck and Raper (31), Smedley-MacLean and associates (32, 33), Witzeman (34), and Verkade and van der Lee (35), who repeated and extended the *in vitro* work of Dakin, found that, while β-oxidation did occur, oxygen could also become attached at the α and the γ posi tion A more serious objection, from the point of view of the whole animal, was the observation by Deuel and associates (36, 37) that more ketone bodies arose in an

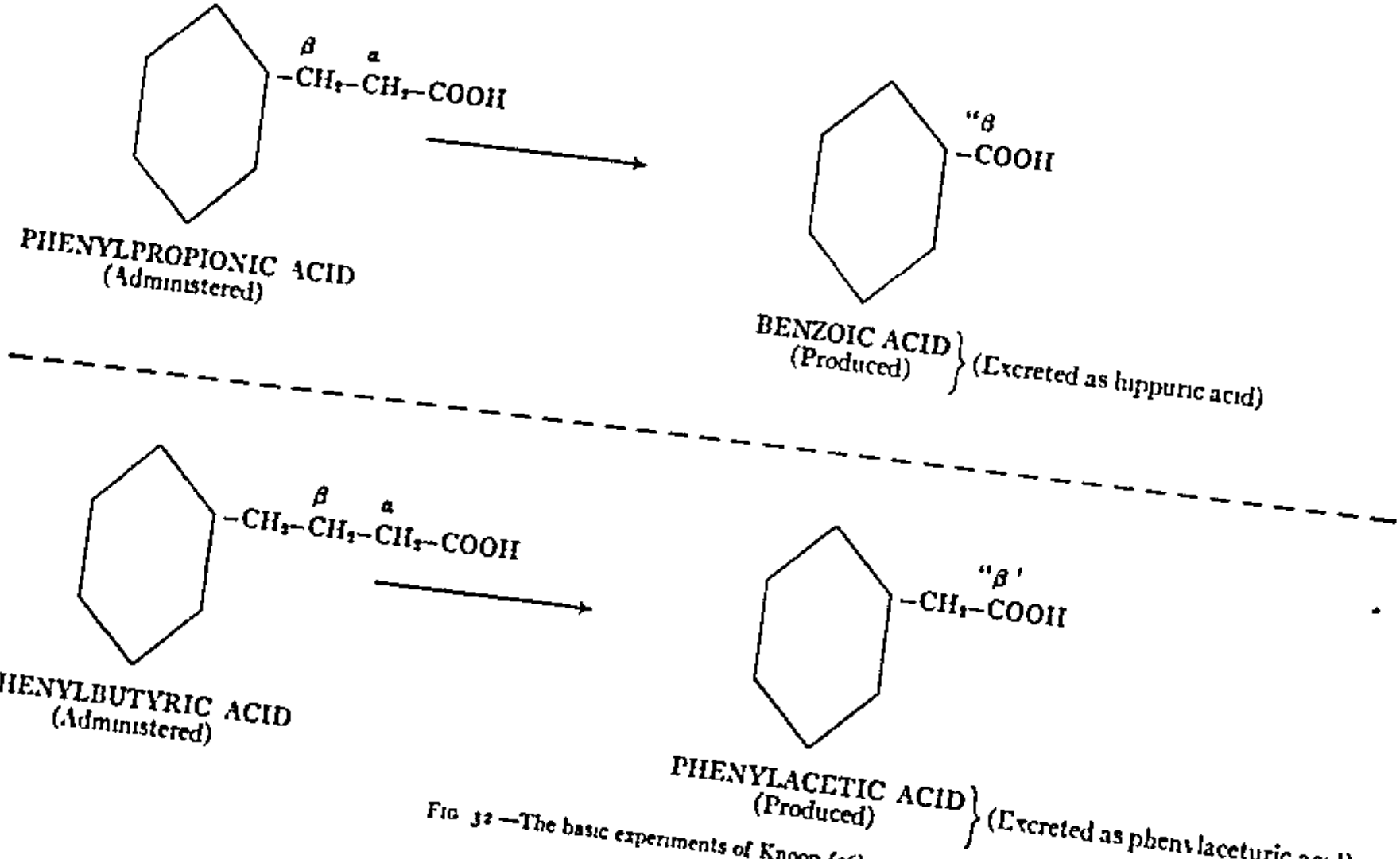


FIG. 32.—The basic experiments of Knoop (26)

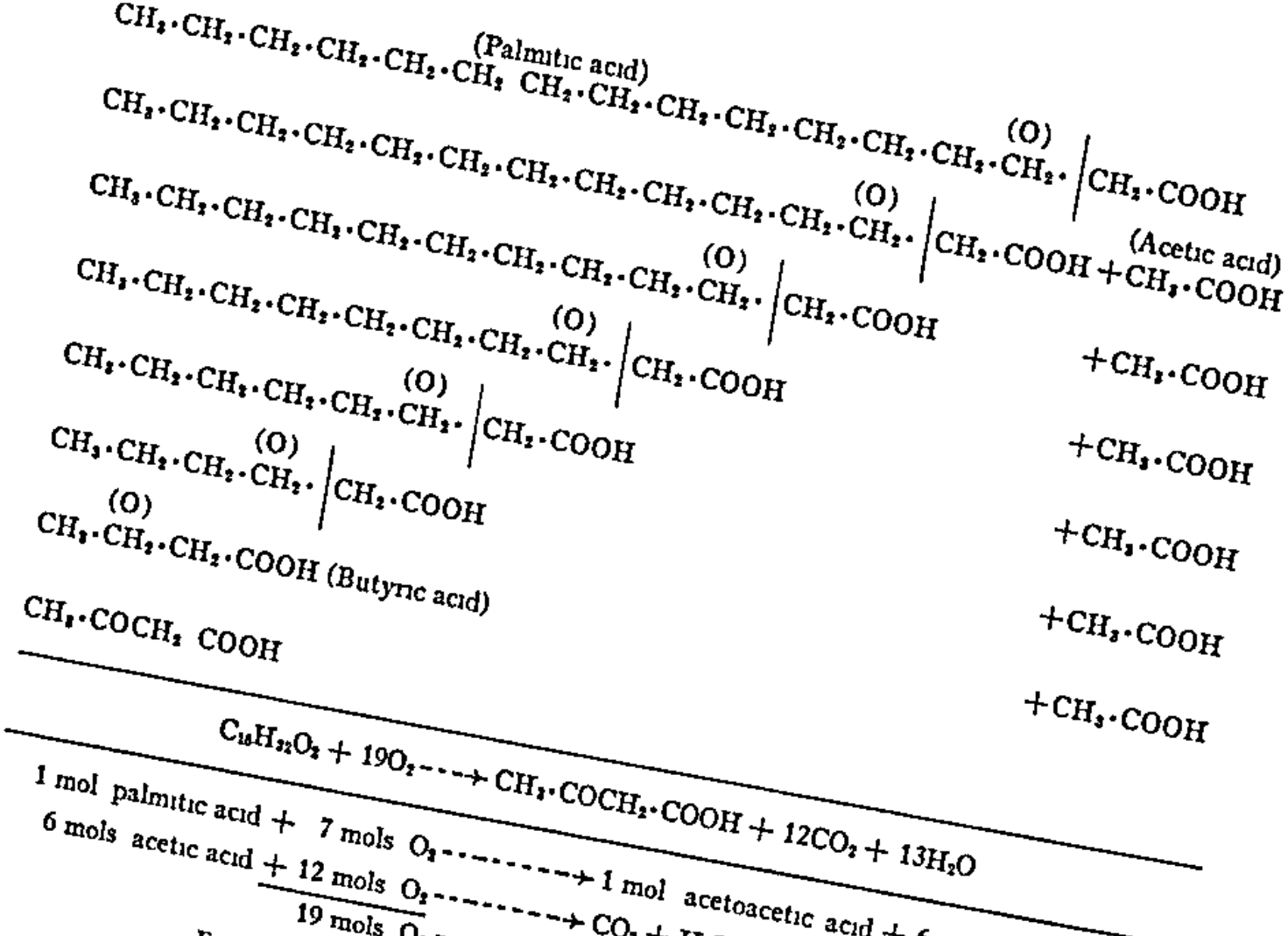
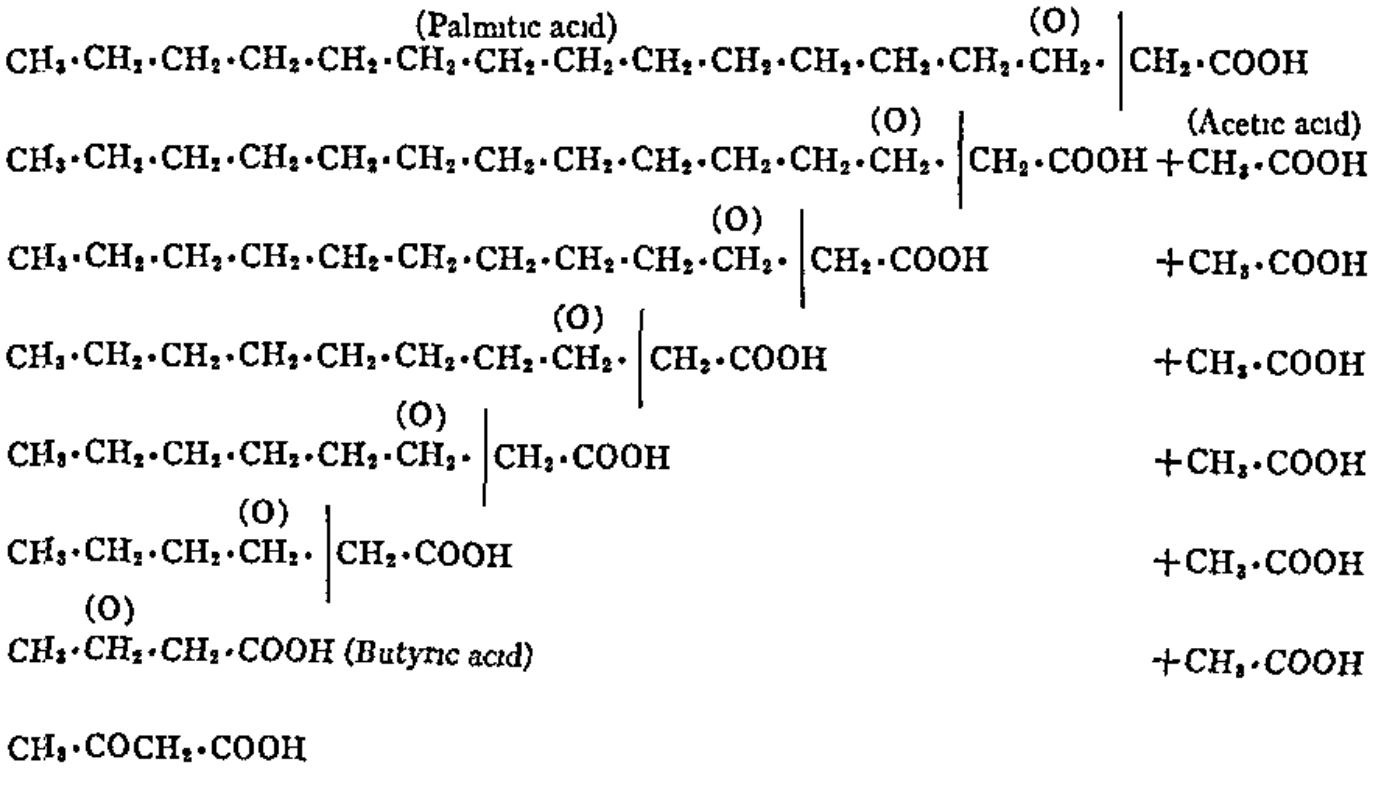


FIG 33.—Knoop's theory of successive β-oxidation (Soskin and Levine [64])

animal fed octanoic acid (C_8) than in an animal fed an equimolar amount of butyric acid (C_4) Shortly afterward, Jowett and Quastel (1, 11), and later Leloir and Muñoz (21), observed that the amounts of ketone bodies formed by liver slices *in vitro* could not be accounted for on the assumption that only the last four carbon atoms of each fatty acid molecule gave rise to a ketone body A similar discrepancy was reported for perfused livers by Blixenkrone Møller (38, 39) and for liver slices *in vitro* by Stadie and co-workers (40) when the oxygen consumption during experiments was compared with that which would have been expected on the basis that all but the last four carbon atoms of each fatty acid was being disposed of by the oxidation of the acetic acid formed The observed oxygen consumptions were far smaller than would allow for this mode of fatty acid breakdown Finally, the improved technics for ketone estimation, which have made possible the determination of relatively small amounts in blood and tissue, have led to the recent finding that the odd numbered fatty acids also give rise to smaller but significant amounts of the ketone bodies, as compared with the even numbered fatty acids This has been reported by Jowett and Quastel (1, 11), Edson (41), and Leloir and Muñoz (21) for isolated tissue (liver) and by MacKay and associates (42) for the intact animal

It is obvious that the hypothesis of successive β-oxidation in the aforementioned form is no longer tenable Indeed, as long ago as 1916, Hurtley (30) proposed the theory of multiple alternate oxidation to account for his failure to find butyric and acetic acids in ketone producing livers He expressed the opinion that the intact fatty acid chain was first oxidized at each alternate carbon atom and then split into blocks of four carbon atoms each—a process which would not necessitate even the transient presence of either of the substances for which he tested According to this hypothesis, the number of ketone molecules arising from a fatty acid would be the whole portion of the quotient when the number of carbon atoms in the fatty acid molecule is divided by 4 This hypothesis was adopted by Deuel, Quastel, Leloir, Blixenkrone Møller, and Stadie, since it accounted for the greater than 1 1 ratio of ketogenesis from the higher fatty acids, the lower oxygen consumption than that expected from the 1 1 ratio, and the formation of ketone bodies from odd numbered fatty acids (Fig 34)

Until recently the multiple alternate oxidation theory was adequate to explain the available data However, it implied a phenomenon rather difficult to explain on *biochemical grounds The simultaneous oxidation of every alternate carbon* atom offered no difficulty But how could one explain the selective splitting of the molecule at every second keto group instead of at every keto group? This difficulty is avoided by a newer conception, which also accounts for other recent evidence not compatible with the theory of multiple alternate oxidation In a systematic *in vitro* study of the ketogenic properties of fatty acids consisting of from one to eleven carbon atoms Jowett and Quastel (1, 11) noted, among other things, ketone pro



$$C_{15}H_{32}O_2 + 19O_2 \longrightarrow CH_3\cdot COCH_2\cdot COOH + 12CO_2 + 13H_2O$$

1 mol palmitic acid + 7 mols O_2 ⟶ 1 mol acetoacetic acid + 6 mols acetic acid

6 mols acetic acid + 12 mols O_2 ⟶ $CO_2 + H_2O$

19 mols O_2 per mol of acetoacetic acid

FIG. 33.—Knoop's theory of successive β-oxidation (Soskin and Levine [64])

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(Palmitic acid)

(O) (O) $CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2$	(O) (O) CH_2 $CH_2 \cdot CH_2$ $CH_2 \cdot$	(O) (O) $CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot$	(O) $CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$
$CH_3 \cdot CO$ $CH_2 \cdot COOH$	$CH_3 \cdot CO \cdot CH_2 \cdot COOH$	$CH_3 \cdot CO \cdot CH_2 \cdot COOH$	CH_3 $CO \cdot CH_2 \cdot COOH$

$$C_{16}H_{32}O_2 + 7O_2 \dashrightarrow 4CH_3 \cdot COCH_2 \cdot COOH + 4H_2O$$

1 mol palmitic acid + 7 mols O_2 ---+ 4 mols acetoacetic acid

(No butyric or acetic acid appears at any stage of the reaction)

1 75 mols O_2 per mol of acetoacetic acid

FIG 34 —Hurtley's theory of multiple alternate oxidation (Soskin and Levine [64])

duction from valeric acid (C_5) and a greater production of ketones from hexanoic acid (C_6) than from butyric (C_4) Since valeric acid is known to give rise to sugar through propionic acid, one can account for the ketone formation only by assuming a condensation of a two-carbon atom fragment from one molecule of valeric acid with a similar two-carbon atom fragment from another molecule The condensation of such two-carbon atom fragments (acetic acid) could also account for the greater ketone formation from hexanoic than from butyric acid Leloir and Muñoz (21) confirmed the findings of Jowett and Quastel

MacKay and co-workers (42, 43) recently performed feeding experiments on intact animals, the results of which supported the interpretation of the above *in vitro* work and led them to postulate a new theory, which they have termed the "β oxidation acetic acid condensation hypothesis" They found, in brief, that the feeding of propionic acid to their animals led to an accumulation of glycogen in the liver without formation of ketone bodies The feeding of valeric acid (C_5) led to both glycogen and ketone body formation Heptanoic acid (C_7) gave rise to glycogen and to more ketones than did valeric acid MacKay and associates postulated that all fatty acid chains whether odd or even numbered, were subjected to oxidation at each alternate carbon atom However, the molecules then split at every keto group to form a number of acetic acid molecules except where a three carbon atom fragment remained to form propionic acid (This, of course, resembles in part the original β oxidation theory, although there is little basis for decid ing between successive or simultaneous oxidation and splitting) Ketones are formed by the condensation of two molecules of acetic acid (Fig 35), a process which has been known since the days of Friedmann (44)

Friedmann's observation was made on isolated livers perfused with solutions containing acetic acid Recently Barnes *et al* (45), using acetic acid containing heavy carbon in *in vitro* experiments, conclusively demonstrated this chemical reaction Weinhouse *et al* (46) carried this type of evidence a step further, using octanoic and butyric acids containing radioactive carbon in the carboxyl groups They found that liver slices converted these substances into acetoacetic acid possessing radioactivity in the β keto group as well as in the terminal carboxyl group This is conclusive evidence that the acetoacetic acid is formed from two-carbon atom fragments

The hypothesis of MacKay and co workers is the most reasonable explanation of the known facts at the present time

REGULATION OF THE KETONE BODIES

For practical purposes the liver may be regarded as the chief, if not the only, source of ketone bodies in the intact organism The extent to which ketones accumulate in the blood or are excreted in the urine will, of course, depend on whether they can be disposed of by the extrahepatic tissues and how rapidly such utiliza

(Palmitic acid)

(O) $CH_3 \cdot CH_2 \cdot$	(O) $CH_2 \cdot CH_2 \cdot$	(O) $CH_2 \cdot CH_2 \cdot$	(O) $CH_2 \cdot CH_2 \cdot$	(O) $CH_2 \cdot CH_2 \cdot$	(O) $CH_2 \cdot CH_2 \cdot$	(O) $CH_2 \cdot CH_2 \cdot$	(O) $CH_2 \cdot COOH$
$CH_3 \cdot COOH$	$CH_3 \cdot COOH$	$CH_3 \cdot COOH$	$CH_3 \cdot COOH$	$CH_3 \cdot COOH$	$CH_3 \cdot COOH$	$CH_3 \cdot COOH$	$CH_2 \cdot COOH$ (Acetic acid)
$CH_3 \cdot CO \cdot CH_2 \cdot COOH$		$CH_3 \cdot CO \cdot CH_2 \cdot COOH$		$CH_3 \cdot CO \cdot CH_2 \cdot COOH$		$CH_3 \cdot CO \cdot CH_2 \cdot COOH$	

$$C_{16}H_{32}O_2 + 7O_2 \dashrightarrow 8C_2H_4O_2 \dashrightarrow 4CH_3 \cdot COCH_2 \cdot COOH + 4H_2O$$

1 mol palmitic acid + 7 mols O_2 $\dashrightarrow$ 8 mols acetic acid

8 mols acetic acid $\dashrightarrow$ 4 mols acetoacetic acid

1 75 mols O_2 per mol of acetoacetic acid

FIG 35 —MacKay's theory of β-oxidation–acetic acid condensation (Soskin and Levine [64])

tion may occur Some of the earlier investigators regarded the ketone bodies as abnormal intermediary products of fat metabolism, which appeared only when there was a failure in carbohydrate oxidation It was thought that under these circumstances the ketones could not be metabolized because of the supposed absence of a coupled oxidation phenomenon which ordinarily occurred (47) It is now well recognized that ketosis occurs under conditions in which large amounts of carbohydrate are being oxidized, and, indeed, it has been impossible to demonstrate any relation between the degree of ketosis and the rate of carbohydrate oxidation (48, 49, 50, 51) On the other hand, there is ample evidence that both acetoacetic acid and β hydroxybutyric acid are catabolized to CO_2 and H_2O by kidney, muscle, heart, brain, testis, etc , as tested on isolated slices *in vitro* (52, 53, 54, 55) Similar evidence is available for perfused whole organs, such as muscles or kidneys (53, 54) The probable pathway of dissimilation of the ketones is indicated in Figure 18 (p 54)

The rate of utilization of the ketone bodies by the normal intact organism has been estimated by a number of investigators (55, 56) It is important to note that this utilization, at the blood concentrations of ketones ordinarily found in clinical ketosis, may constitute a highly significant portion of the total energy requirements of the organism Indeed, it has been estimated that ketone utilization in the animals which have been studied could account for from 50 to 80 per cent of the total oxygen consumption In view of this great capacity for the utilization of ketones, the small amounts normally found in the blood may indicate that even the normal liver forms, and continues to secrete, some ketone bodies into the blood [1]

It might be supposed, however, that the severe ketosis of diabetes, phlorhizin poisoning or starvation is the result of some difficulty in the utilization of ketones by the periphery, with or without a greater production by the liver This possibility has been tested both *in vitro* and *in vivo*, without confirmation Chaikoff and Soskin (14) have shown that the peripheral tissues of the diabetic organism dispose of the ketone bodies as rapidly as do those of the normal animal This has since been amply confirmed (25 48, 51, 54) With the possible exception of the adrenalectomized animal (58) it must be assumed that, whenever ketones appear in excess in the blood and other tissues this condition is due to a rate of formation and secretion by the liver sufficiently rapid to exceed even the large disposal capacity of the periphery It is thus no longer proper to speak of antiketogenesis in the sense so long employed by clinicians, by which they actually meant ketolysis (ketone oxidation) In view of present knowledge, the various ketogenic antiketogenic ratios (47) which have been used to calculate the amounts of carbohydrate "necessary for the oxidation of the ketone bodies ' must be regarded as being without any real significance

[1] Crandall and his co workers (57) differ from this opinion on the basis of experiments with the London cannula technic

will be a diminution of ketogenesis—even though some of these substances themselves be ketogenic in action if given at a time when the enzyme syster unoccupied Such substances are odd numbered fatty acids, certain amino and benzoic, cinnamic, and α aminobutyric acids The type of inhibition they exert is somewhat analogous to the well known action of malonate succinodehydrogenase system (62)

We may summarize by saying that the ketone bodies are probably normal mediates of fatty acid catabolism in the liver They appear in excess in the whenever the hepatic metabolism of fat is sufficiently speeded up, either by of carbohydrate substrate or by a disturbance in the normal regulation of th strate mixture The ketone bodies are readily utilized by the peripheral ti under practically all known conditions The utilization of ketone bodies may some relationship to the utilization of sugar by the extrahepatic tissues, in so these two substrates may compete for the available oxidative mechanisms it is evident that the development of a ketosis in the diabetic state cannot l garded as evidence for the non utilization theory of diabetes It is perhaps compatible with the overproduction theory, for if one broadens the latter co tion to signify the overproduction of metabolic substrates (i e , sugar plus ket it is clear that the use of the ketones by the peripheral tissues will leave a gr excess of sugar to accumulate in the blood and spill over into the urine

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Data

Urine nitrogen	0 202 gm/hr
O_2 consumption	11 195 L/hr
CO_2 production	8 290 L/hr

Calculations

1 gm of urine N represents 6 25 gm of metabolized protein

$$\text{Protein oxidized} = 0\ 202 \times 6\ 25 = \underline{1\ 26\ \text{gm/hr}}$$

To oxidize 1 gm of protein 0 957 L of O_2 are required and 0 774 L of CO_2 are produced

O_2 used in the oxidation of protein = 1 26 × 0 957 = 1 206 L

and CO_2 produced in the oxidation of protein = 1 26 × 0 774 = 0 975 L

Non protein O_2 = 11 195 − 1 206 = 9 989 L

and non protein CO_2 = 8 290 − 0 975 = 7 315 L

$$\text{Non protein R Q} = \frac{7\ 315}{9\ 989} = \underline{0\ 733}$$

Percentage of non protein O_2 used by CHO =

$$100\left(\frac{0\ 733 - 0\ 707}{1\ 00 - 0\ 707}\right) = 8\ 87 \text{ per cent}$$

$$O_2 \text{ used for CHO oxidation} = \frac{9\ 989 \times 8\ 87}{100} = 0\ 886 \text{ L}$$

and CO_2 produced by CHO oxidation (R Q = 1 00) = 0 886 L

O_2 used for fat oxidation = 9 989 − 0 886 = 9 103 L

and CO_2 *produced by fat oxidation* = 7 315 − 0 886 = 6 429 L

To oxidize 1 gm of CHO (starch) 0 829 L of O_2 are required

$$\text{CHO oxidized} = \frac{0\ 886}{0\ 829} = \underline{1\ 07\ \text{gm/hr}}$$

To oxidize 1 gm of fat 2 013 L of O_2 are required

$$\text{Fat oxidized} = \frac{9\ 103}{2\ 013} = \underline{4\ 52\ \text{gm/hr}}$$

Similar calculations may be made for all levels of the N P R Q from 0 7 to 1 0 In actual practice, it is customary to ascertain the significance of an R Q determination by consulting tables or nomograms prepared by Zunz and Schumburg (8), Du Bois (9), and others (5)

THE COMPOSITE NATURE OF THE R Q

It is becoming increasingly more evident that the N P R Q of the whole body, like the D N ratio, cannot be regarded as the index of a single process The orthodox interpretation of the N P R Q of about 0 7 involves the tacit assumption that the only vital processes (aside from protein catabolism) which are in progress and which ultimately consume oxygen and give rise to CO_2 are those associated with the oxidation of fat Yet there is very satisfactory evidence that other processes which require oxygen or yield CO_2 are taking place under those conditions It is generally agreed, for example, that the brain derives its energy solely at the expense of carbohydrate and yields an R Q of about 1 0 at all times (10, 11, 12 13 14) This high R Q must be balanced by a correspondingly low one in some other tissue or organ if the composite R Q of 0 7 obtained from the whole body is to

mean anything at all Authentic low R Q 's below o 7 have been obtained particu larly from the liver, as will be discussed in chapter xiii (p 142) It is, therefore obvious that the correct interpretation of an R Q cannot be as simple as that used by its original exponents and some of their present day followers

The conception of constituent R Q 's going to form a composite R Q has actual ly been used to explain values of the R Q over 1 o The transformation of carbo-hydrate into fat, a material with relatively lower oxygen content, would yield a theoretical R Q of about 8 o

$$4C_6H_{12}O_6 + O_2 \rightarrow C_{16}H_{32}O_2 + 8CO_2 + 8H_2O$$
$$R\,Q = \tfrac{8}{1} = 8\,0$$

This transformation usually occurs when there is a plethora of carbohydrate avail able in the body Under these circumstances the R Q above unity is said to result from the transformation and the simultaneous oxidation of carbohydrate (5, 7) However, for the sake of convenience, this type of explanation has been confined artificially to R Q values over 1 o It is evident that, if carbohydrate could be converted to fat under conditions where fuels other than carbohydrate were also being oxidized any R Q under 1 o might have a high component due to the transformation, thus abrogating the classical calculations In reality, there is no evidence that this does not occur In fact, the work of Schoenheimer and his asso ciates (15 16), in which heavy isotopes were used as markers, has clearly indicated that there is a constant interconversion of one foodstuff into another even under conditions where no body weight is gained or lost

Cathcart and Markowitz (17) and others have shown that the oral administra tion of 50 gm of glucose to the fasting human causes a leisurely rise in the R Q to values somewhat less than 1 o, while the administration of equivalent quantities of sucrose, galactose, levulose, or dihydroxyacetone causes a prompt rise in the R Q to values above unity The more rapid rise in the R Q which occurs with the latter substances cannot be accounted for by their relative rates of absorption from the gastro intestinal tract, and their chemical composition is theoretically incom patible with an R Q over 1 o It is clear, therefore, that even such relatively simple foodstuffs do not yield R Q s which may be reasonably interpreted as re sulting from their oxidation alone

Much has been made of the fact that the R Q of the whole mammalian organ ism has not very often been found to fall below o 7 Indeed, it was formerly cus *tomary to ascribe any lower R Q to some undetected fault in technic More re* cently, admittedly authentic low R Q s have been obtained (18, 19), and other instances in the literature which are similarly free from technical criticism (19) have been reviewed Some of these low values were obtained in normal human sub jects under special conditions of feeding—for example, on high fat intakes before

the subjects became acclimatized to the abnormal diet This is significant because the customary feeding habits of man and of animals have resulted in rather arbitrary conventions as to the number, composition, and size of meals and as to the periods during which R Q measurements of the absorptive and post absorptive states are made The intake of food is ordinarily spread over a considerable proportion of the 24 hours This means that all the various oxidations, conversions etc, which yield the highest and lowest components of the composite R Q are usually proceeding simultaneously Under these circumstances one could hardly expect to obtain anything more than an intermediate range of values for the R Q of the whole body

To succeed in demonstrating a truer range for the component R Q s of the body on a normal diet, it would be necessary to set the experimental conditions so as to allow the processes responsible for either the lowest or highest component R Q s to predominate temporarily In other words, it would be necessary "to catch the metabolic processes off balance" This has been done by Werthessen (20), who trained rats to eat their entire 24 hour food requirement within a period of 1–5 hours He found that in the same animal, after such a meal, the R Q (determined at frequent intervals) varied from extremely low to extremely high values The range of these variations in all his animals was from 0 27 to 1 70! (See Fig 37) Markowitz (personal communication), working with Cathcart, performed this experiment upon himself and obtained results similar to those reported by Werthessen These experiments show that the range of R Q values ordinarily obtained depends not so much upon the chemical reactions in the body as upon the customary conditions of observation The extreme R Q values obtained under special conditions again demonstrate that the usual R Q s are integrals of higher and lower quotients

The fact that the R Q of the whole body is a composite of many R Q 's originating in different organs and arising from different chemical reactions occurring simultaneously, does not preclude the possibility that all the energy involved may not ultimately be derived from a single foodstuff When an N P R Q of 0 7 is obtained, it is possible that only fat is being broken down, that some of it is oxidized directly in one organ, that in a second organ another portion of the fat is transformed into other metabolites and that these metabolites are oxidized in still a third one The net result of all these processes could still be an R Q of 0 7 The point is that this figure, by its very nature, depends solely on the starting material and the end products of the series of reactions It gives no indication whatever of the intermediate reactions Under these circumstances the characteristic diabetic R Q cannot be interpreted as indicating a lack of ability to oxidize carbohydrate Thus, a fatty acid might break down directly to CO_2 and H_2O as follows

$$C_{18}H_{36}O_2 + 26O_2 \rightarrow 18CO_2 + 18H_2O$$

The theoretical R Q of this process is 18 — 26 = 0 693 The same fatty acid might first be converted to carbohydrate and then oxidized

$$C_{18}H_{36}O_2 + \underline{8O_2} \rightarrow 3C_6H_{12}O_6 + \underline{18O_2} \rightarrow 18CO_2 + 18H_2O$$

The R Q for this manner of breakdown is also 18 ÷ (18+8) = 0 693

A further characteristic of the diabetic R Q is its failure to rise after the administration of carbohydrate as it does in the normal organism This abnormality may be explained on exactly the same basis as the quantitative excretion of ad

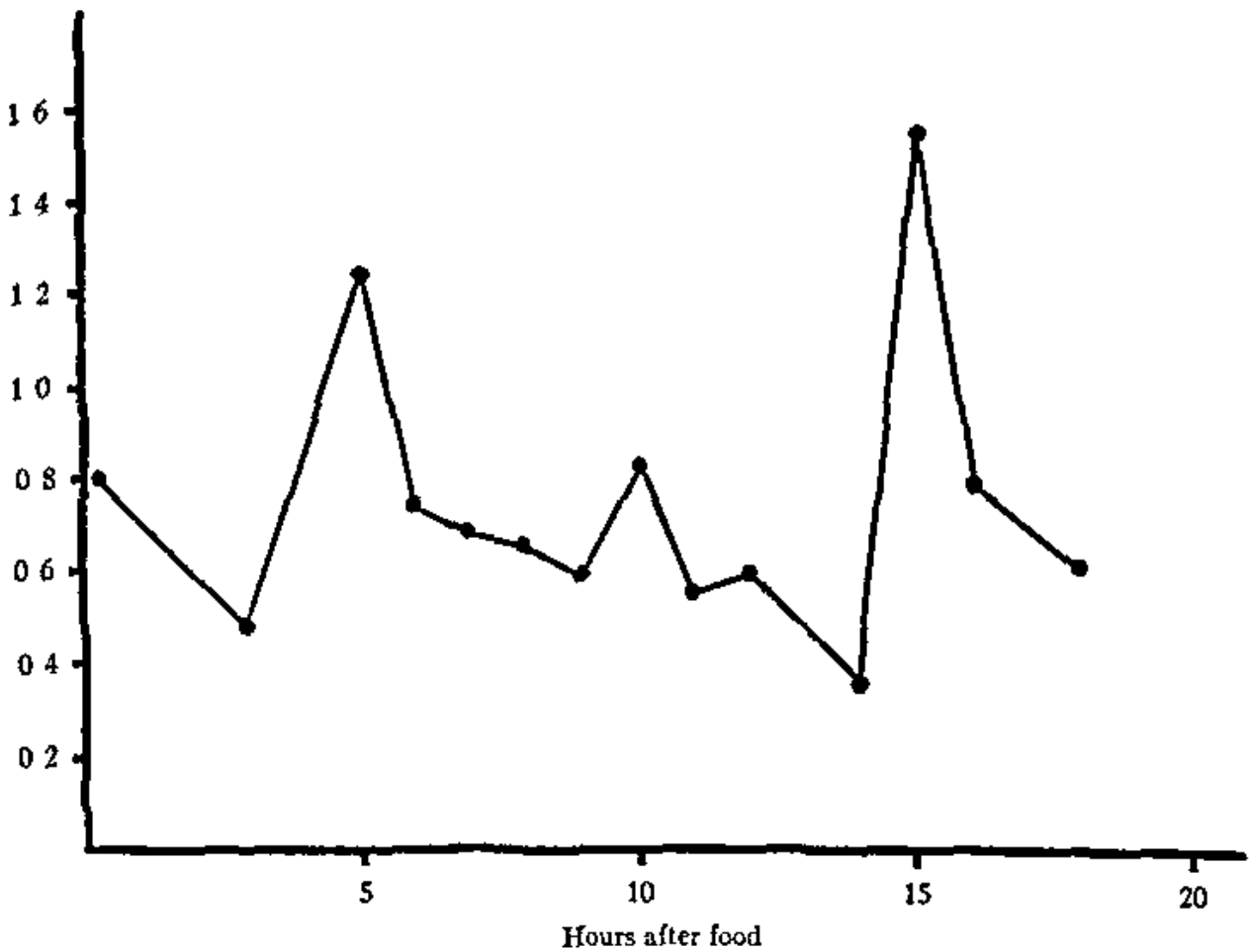

FIG 37 —Serial determinations of the R Q in a trained rat following the intake of its 24 hour food requirement at a single meal (From Werthessen [20])

ministered sugar which we have previously discussed (p 105) It is due to the fact that the extrahepatic tissues of the diabetic organism are already being supplied with a superabundance of sugar so that the administered carbohydrate is not metabolized but overflows into the urine together with the excess arising from the animal s own liver

It is clear that neither the low R Q of diabetes nor the failure of the R Q to rise following the administration of sugar constitute evidence for a lack of ability to oxidize carbohydrate (For a further discussion of the R Q see chap XIII)

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CHAPTER XII

GLUCONEOGENESIS FROM PROTEIN

THE discussion of the D N ratio (chap ix) led to the conclusion that the type of evidence obtained by feeding protein to the depancreatized animal shows only that some of the sugar which is excreted is derived from the administered protein, and that it is impossible to say to what extent this conversion occurs When the phlorhizinized animal is used in the same way, there is the added difficulty of having to account for a relatively larger sugar excretion than that which occurs in the depancreatized animal

Somewhat simpler experimental conditions are possible when perfused organs and isolated tissues are used Since the composition of proteins is variable, the testing of individual amino acids on the isolated organs and tissues is a further simplification of the problem The use of amino acids is convenient as regards their addition to perfusates and nutritive media, and the results are quite acceptable as reflecting normal physiology, for both ingested proteins and endogenous proteins are hydrolyzed to amino acids in the intact organism before further catabolism

The literature up to the year 1930 relating to the conversion of amino acids to carbohydrate was comprehensively reviewed by Rapport (1) Table 15 summarizes the essential information compiled by him and the additional evidence which has accumulated during the intervening years Data on the conversion of amino acids to β keto acids are also included because of the possible transformation of the latter into sugar, a subject to be discussed in the following chapter The information in Table 15 is derived from the following types of experiments

In vivo

1 Amino acids are fed to depancreatized or phlorhizinized dogs and the urine is analyzed for the extra glucose excreted over and above the amounts excreted on previous days

2 Amino acids are fed to starving normal animals, and the rise in liver glycogen is used as an index of transformation to carbohydrate An increase of the ketone bodies in the blood and urine is taken as evidence of conversion of the amino acids to β keto acids

Perfusion experiments

1 The liver is perfused with blood to which the various amino acids are added A rise in the glucose or ketone content of the perfusing blood is taken as evidence for transformation

TABLE 15*

AVAILABLE EVIDENCE FOR GLUCONEOGENESIS AND KETOGENESIS FROM THE AMINO ACIDS

Amino Acid	*In vivo* Experiments			Perfusion and *in vitro* Experiments		
	To Carbohydrates	To Ketones	References and Remarks	To Carbohydrates	To Ketones	References and Remarks
Glycine	+	o	Lusk (14), phlorhizinized dogs	o		Bach (5), liver and kidney perfusions and slices
	o		Pflueger (15), normal dogs			
	o		Wilson (16), normal rats	o		Bach (6), liver slices
	o→+	o	Butts (17), normal rats			
	+		MacKay (12), normal rats			
	o		Olsen (11), normal rats (isotope carbon as tracer)			
Alanine	+	o	Lusk (14), phlorhizinized dogs	+		Embden (18), liver perfusion
	+	o	Butts (17), normal rats	+		Krebs (7), liver slices
	+	o	Wilson (16), normal rats			
Serine	+		Rapport (1), phlorhizinized dogs	+		Chargaff (19), liver extracts
	+	o	Butts (17), normal rats			
Valine	o	o	Dakin (20), phlorhizinized dogs			
	o→+	o	Butts (21), normal rats			
	+		Rose (22) phlorhizinized dogs			
Leucine	o	+	Butts (23), normal rats	o	+	Embden (24), liver perfusion
	o		Dakin (20), phlorhizinized dogs		+	Edson (13), liver slices
Isoleucine	+	+	Butts (23), normal rats			
	o	o	*Dakin (25), phlorhizinized* dogs			
Norleucine	+	o	Butts (23), normal rats			
Aspartic	+	o	Lusk (14), phlorhizinized dogs	+		Krebs (7), liver and kidney
	+	o	Butts (26), normal rats			
Glutamic	+	o	Lusk (14), phlorhizinized dogs	+		Weil Malherbe (27), liver
	+		Wilson (16), normal rats			
	+	o	Butts (26), normal rats			
Arginine	+	o	Dakin (20), phlorhizinized dogs			
	o→+		Butts (28), normal rats			
Ornithine	+	o	Dakin (20), phlorhizinized dogs			
Lysine	o	o	Dakin (25), phlorhizinized dogs			
	o	o	Butts (28) normal rats			
Cysteine	+		Dakin (25), phlorhizinized dogs	+		Smythe (29), liver slices
Cystine	o		Butts (30), normal rats	+		Smythe (29), liver slices
Methionine			Transformed to cystine (*q v*)			
Phenylalanine	o	+	Dakin (20), phlorhizinized dogs		+	Embden (24), liver perfusion
	+	o	Butts (31, 32), normal rats		+	Edson (13), liver slices
Tyrosine	o	+	Lusk (14), phlorhizinized dogs		+	Embden (24), liver perfusion
	o→+	o	Butts (31, 32), normal rats		+	Edson (13), liver slices
Histidine	o	o	Dakin (25), phlorhizinized dogs			
	+	o	Remmert (33) and Feather stone (34), normal rats			

* Zero indicates negative experimental results indicates no data

TABLE 15—*Continued*

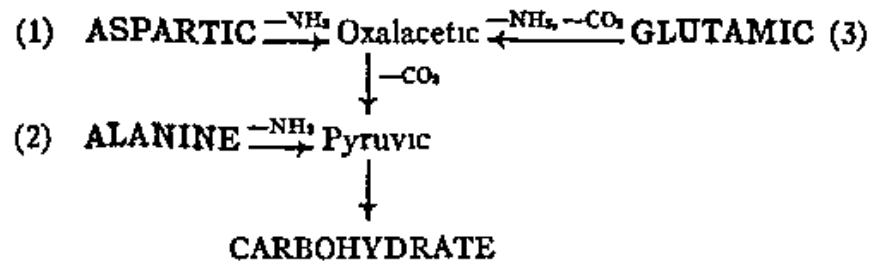
Amino Acid	*In vivo* Experiments			Perfusion and *in vitro* Experiments		
	To Carbohydrates	To Ketones	References and Remarks	To Carbohydrates	To Ketones	References and Remarks
Tryptophane	o	o	Dakin (25), phlorhizinized dogs			
	o	o	Borchers (35), normal rats			
Proline	+	o	Dakin (25) and		o	Edson (13), liver slices
	+	o	Kapfhammer (36), phlorhizinized dogs			
Hydroxyproline	+	o	Kapfhammer (36), phlorhizinized dogs		+	Edson (13), liver slices

In vitro

1 Tissue slices (generally liver) are incubated in the Warburg respirometer with various amino acids, and the rise in total carbohydrate, carbohydrate intermediates, and ketone body content of the slices is measured

2 Enzyme preparations from animal tissues are employed to follow the pathway of the intermediate metabolism of amino acids

It may be seen that a large part of the evidence collected in Table 15 was obtained *in vivo*, using the D N ratio or the increase in liver glycogen content as the criterion for carbohydrate formation The same objections as were raised against the use of the D N ratio in the study of gluconeogenesis from protein also apply in the present connection The increase in liver glycogen after amino acid administration was not regarded as a quantitative index, even by those who used this criterion This leaves the perfusion and the *in vitro* experiments as the possible source of reliable quantitative information When all the quantitative evidence is summarized, it may be seen that definite information is available about only six amino acids Alanine, aspartic acid, and glutamic acid are converted to carbohydrate in definite proportions and by known pathways, as follows



Lysine, tryptophane, and leucine are not converted to any measurable degree. There our quantitative information ends

This leaves fifteen amino acids about which only qualitative information is available, and the information we do have casts considerable doubt upon the validity of even this type of conclusion For example, *the in vivo evidence as to* gluconeogenesis from glycine is contradictory, only two out of seven sets of experimenters having obtained apparently unequivocal evidence that this occurred The *in vitro* evidence as to the metabolic fate of glycine is not wholly clear, and it is contradictory in some respects It is well established that glycine is one of the building stones of creatine (2, 3, 4) and that it may condense with α ketoacids probably forming new amino acids (5) However, there is no unanimity of opinion as to the deamination of glycine Thus, Bach (5, 6) found that neither kidney nor liver slices were able to deaminate glycine Moreover, the standard amino acid oxidase preparations exert no effect upon this amino acid (7) However, very recently Green *et al* (8) prepared a glycine oxidase system from kidney which converts glycine to glyoxylic acid

$$CH_2NH_2 \quad COOH \xrightarrow{+IO_2} CHO \quad COOH + NH_3$$

Another enzyme system converts glyoxylic acid to oxalic acid (COOH COOH) (8), but, since previous work has shown that oxalic acid is not further convertible in the animal body (9, 10), the work of Green indicates that glycine does not by itself give rise to glucose

This conclusion is strengthened by the work of Olsen *et al* (11), who fed isotopic glycine to rats The liver glycogen showed a delayed rise (confirming Mac Kay [12]), but this glycogen was not derived from the administered glycine, for it did not contain any of the heavy carbon Olsen *et al* (11) drew the important conclusion that evidence concerning the conversion of amino acids to glucose derived from *in vivo* and *in vitro* experiments should be re-examined, using labeled amino acids It is not sufficient to show extra glucose excretion or increased liver glycogen *To be unequivocal, the evidence must show that the newly formed* glucose or glycogen is built up from the constituent atoms of the amino acid under investigation

To cite another example, proline administered to phlorhizinized dogs has been [illegible] on that it is (a change (13)

We may summarize the present knowledge by saying that, whatever its empirical usefulness, *the figure of 44–58 per cent commonly used in* metabolic and nutritional work to calculate the carbohydrate equivalent of protein has no real basis in fact Even under the simplest conditions, using amino acids and the *in vitro* technic, it has thus far been possible to ascertain the quantitative fate of only *a few of* the amino acids It is evident that much work remains to be done in this field

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tomy experiments for the sugar utilization of the extrahepatic tissues of normal and depancreatized animals, calculated that sugar must be formed from fatty acids in the livers of both types of animal, as follows (9)

hour, i e 60 gm per day for a 10 Kg dog The average arteriovenous blood difference in fasting animals is 4 mg per cent [Corn (5)] and if the tissues of a fasting 10 Kg dog are absorbing sugar

10 Kg dog is actually

or so of fasting the source of 60 − (25 + 6 + 6) = 23 gm of the daily hepatic sugar production of the 10 Kg dog is unaccounted for This discrepancy is so great that it seems impossible to account for the facts without assuming considerable conversion of fatty acid to sugar in the liver of the fasting dog

Depancreatized dog —Mann's [11] observation that hepatectomy of a previously depancrea

producing at least 45 gm of sugar per diem for a 10 Kg dog and a loss of

D N ratio of

r resulted from

sugar a mani

DIRECT EVIDENCE

23) Our present knowledge of tissue-enzyme chemistry and of intermediary metab

olism indicates the existence of suitable pathways for gluconeogenesis from fatty acids (chap iii, p 54) The point at issue, therefore, is not whether the process can occur but whether it does occur in the mammalian organism

In view of Young's calculations, it is of interest to consider why the administration of fat to experimentally diabetic animals has usually not resulted in sufficient excretion of extra sugar to indicate gluconeogenesis from fatty acids when the calculations were made on the basis of the classical interpretations of the D N ratio (24) This is not surprising when it is remembered that these interpretations, by their very nature, practically exclude the possibility that such calculations might yield positive results Even so, it might still be possible to show extra sugar excretion if the experimental animal could make additional amounts of sugar over and above that which it is already forming from endogenous protein and fat, *including the amount which is being utilized during the experiment* But this involves the unwarranted assumption that the capacity of the liver for gluconeogenesis from fat has not been reached before the fat is administered The fact that this is not the case for protein has no bearing for it happens that fat is the only stored food substance present in practically unlimited amounts so far as the daily requirement of the body is concerned It might therefore be expected that fat would be used to capacity when the liver is forming sugar at an uncontrolled rate

From the practical standpoint the experimental procedure to test the extra sugar excretion involves the administration of fat to the diabetic animal on the fourth or fifth day after pancreatectomy after the withdrawal of insulin, or after starting phlorhizination At this time the animal is suffering from acute diabetes with ketosis, and the administered fat makes him even more sick In certain experiments in which some extra excretion of sugar after fat administration was reported (25), the animals died shortly In order to obtain positive results by this method, it is apparently necessary to exceed physiological limitations to a degree incompatible with life

There have been a number of experiments the results of which favor gluconeogenesis from fat even though the investigators did not take into account the factor of utilization In these experiments neutral fat or fatty acids were administered to *intact* normal or diabetic animals, or certain hormones (e g , epinephrin) or drugs (e g , phlorhizin) were given to such animals in an attempt to force excessive gluconeogenesis from endogenous fat stores The results of these experiments were judged by the increases in carbohydrate content of the liver and muscles of the normal animals and by the increased sugar excretion of the diabetic animals As might be predicted from our previous discussions of the dynamic balance and the D N ratio, these experiments have yielded both positive (3 25, 26 27, 28, 29 30, 31) and negative (24 32, 33) results Under the circumstances it is justifiable to place greater weight on the positive than on the negative findings This evidence and preceding work of a similar kind have been comprehensively reviewed by

Macleod (3) and Geelmuyden (2) and will not be discussed here It will be more profitable to confine the discussion to more recent and less controversial evidence

The theoretical R Q for the conversion of protein to carbohydrate has been variously calculated as 0 613 (Magnus Levy [34]), 0 632 (Lusk [35]), and 0 706 (Geelmuyden [36]) The R Q for gluconeogenesis from fat has been calculated to be about 0 28 by Pembrey (37) and by Macleod (3) The theoretical R Q for ketogenesis from fat may be calculated to range from 0 65 to 0 00, depending upon the number of molecules of β hydroxybutyric acid which are supposed to arise from one molecule of fatty acid The work of Blixenkrone-Møller (38) strongly indicates that the value lies closer to zero than to the higher figure

Since gluconeogenesis and ketogenesis occur primarily in the liver, it would be expected that R Q determinations performed on the isolated liver under the appropriate physiological conditions should yield very low values This is the case Gemmill and Holmes (39) found that the R Q of liver slices from a rat fed on a normal diet averaged 0 79 while that from a rat fed butter averaged 0 58 Stadie and co-workers (40) observed R Q 's of about 0 32 in liver slices from the depancreatized cat Similarly in the perfused livers of normal and depancreatized cats Blixenkrone Møller (38) obtained R Q values which averaged 0 57 for the normal

coneogenesis from fat But the simultaneous occurrence of gluconeogenesis from protein, and particularly of variable ketogenesis, makes it difficult to use the R Q as a quantitative index Evidence based upon chemical determination of newly formed carbohydrate or carbohydrate intermediates is more convincing

We have already mentioned the work of Gemmill and Holmes (39), in which they found very low R Q values in the isolated liver slices of butter fed rats They also observed a coincident increase in the carbohydrate content of these slices which was greater than the increase observed in liver slices taken from rats on a

each substance and in practically all tissues they observed a large production of lactic acid The simultaneous decrease in the carbohydrate content of the tissue when it occurred, was significantly less than the increase in lactic acid In the case of the liver, when oxygen was present there was an increase in the carbohydrate content as well as in the amount of lactic acid It was obvious that the lactic acid could not be accounted for as arising from carbohydrate The authors considered the possibility that the added fatty acids might have stimulated the production of

lactic acid from some other substance but they concluded that this supposition could not be justified They pointed out that in the brain and liver for example they were dealing with tissues which ordinarily produce little or no lactic acid and which contain no other known precursor of lactic acid Their work therefore yields convincing evidence for the formation of carbohydrate from fat through a lactic acid stage (probably via pyruvate) More recently gluconeogenesis from fat in isolated mammalian tissue has again been confirmed by Weil Malherbe (43) who demonstrated the *in vitro* formation of sugar from added acetoacetic acid by kidney slices

Another method by which the extrahepatic utilization of sugar has been ex cluded and one which is a step nearer the intact organism is the perfusion of the isolated whole liver This method is not easy and it is sometimes difficult to obtain satisfactory preparations (44) Nevertheless a number of competent investigators have carried out reasonably successful liver perfusions as judged by a maintained rate of flow of perfusate through the liver with little or no edema the continued excretion of bile and the storage of glycogen Burn and Marks (45) perfused the glycogen poor livers of fat fed dogs and of a depancreatized cat A large production of acetone bodies and of sugar was observed The pre-existing carbohydrate con tent of the livers accounted for but a small fraction of the sugar which appeared The disappearance of lactic acid was ruled out as a factor As regards gluconeo genesis from protein Burn and Marks rightly (in view of our previous discussion of the D N) rejected the use of any of the orthodox values for the D N ratio In stead they calculated that if all the carbon in the protein molecule were recom bined so as to form dextrose the ratio of dextrose produced to nitrogen set free in the form of urea and ammonia cannot be greater than 8 3 1 Values for the D N ratio above this figure would therefore demonstrate gluconeogenesis from fatty acid Out of a total of forty seven determinations of the D N ratio thirty two ex ceeded the value of 8 3 and in seven cases the ratio rose above 17 0

Heller devised ingenious methods (46) to observe the sugar output of the liver *in situ* in normal and phlorhizinized cats anesthetized with Pernocton After de ducting the amounts of carbohydrate which might have come from glycogen lac tic acid and glycerol he calculated D N ratios ranging from 5 0 to 18 0 (47)

More complete and conclusive work upon the subject was done by Blixenkrone Møller (48) He perfused the livers of normal and of phlorhizinized cats with sodi um butyrate After accounting for other possible sources of carbohydrate he ob tained D N ratios ranging from 10 0 to 20 0 or over Perfusion with sodium suc cinate yielded D N ratios as high as 42 0 He concluded that about 20 per cent of the added butyric acid was converted into ketone bodies and that the remainder went to sugar via succinic acid Cat livers were perfused with blood according to a technic worked out by the author In control experiments this technic per mitted glycogen storage from glucose etc, thus demonstrating preservation of

normal liver function Chemical determinations included *glycogen, fat, and ke* tone content of the liver before and after the perfusion, blood sugar, ketones lac tic acid, urea, oxygen, and CO_2 at frequent intervals Sodium butyrate was added *to the perfusing blood* after a control period Table 16 shows a typical experiment performed on a liver from a normal cat (48)

It can be seen from Table 16 that the carbohydrate, newly formed in a liver per fused with sodium butyrate, could not have arisen from protein conversion and must have been derived from the fatty acid Unequivocal confirmation of this con version was supplied by Hastings and co workers (49), *who fed butyric acid con* taining "heavy" C atoms to normal rats and found the labeled C in the liver gly

TABLE 16

PERFUSION OF NORMAL CAT LIVER WITH SODIUM BUTYRATE (BLIXENKROVE MØLLER [48])

Liver weight, 61 gm blood volume 300 cc sodium butyrate added 500 mg

CHEMICAL ANALYSIS	CONCENTRATION (MG PER CENT)			AMOUNT FORMED (MG)	REMARKS
	Initial	Final	Difference		
Total ketones of blood	39 3	93 5	54 2	162 6	188 8 mg of ketones appeared
Total ketones of liver	33 0	76 0	43 0	26 2	
Blood sugar	222 0	304 0	82 0	246 0	341 0 mg of carbohydrate appeared
Liver glycogen	80 0	235 0	155 0	95 0	
Blood urea	58 0	70 0	12 0	36 0	Corresponds to the breakdown of 106 0 mg of protein

In control experiments with butyrate 75 mg of ketones and 54 mg of carbohydrate were formed The breakdown of protein could have given rise at the utmost to 100 mg of sugar The balance reveals therefore that the sodium butyrate gave rise to—

(1) 188 8 − 75 0 = 113 8 mg of ketones
(2) 341 − (100 − 54) − 295 0 mg of sugar

cogen of these animals These findings are quite in accord with other pertinent evi dence discussed elsewhere in this volume We have seen that fatty acids are broken down to the ketone bodies by way of acetic acid (chap x) Acetoacetic acid may condense with oxalacetic acid to enter the tricarboxylic acid cycle, which is the common reservoir for derivatives of all three major foodstuffs (56) And each mem ber of the cycle has been shown to be capable of resynthesis to glucose (55) In addition, acetic acid may, under certain circumstances, enter directly into the tricarboxylic acid cycle without going through the acetoacetic acid stage (52) (see chap III, p 54)

Figure 38 graphically summarizes the more direct evidence for gluconeogenesis from fat and indicates the intermediate chemical steps by which it may occur We may conclude that this process can and does play an important role in both the normal and the diabetic mammalian organism

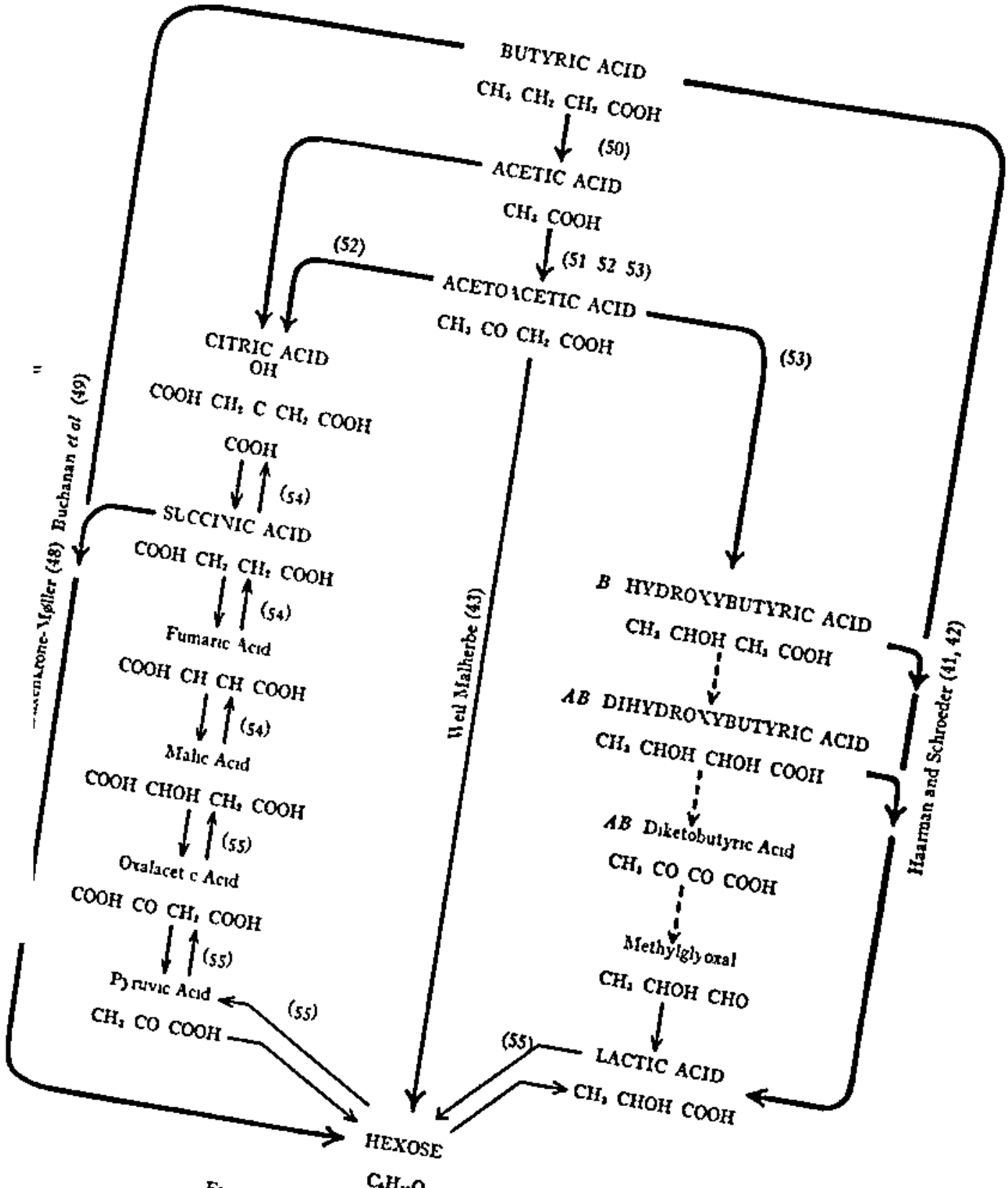

FIG 38 Pathways for gluconeogenesis from fat

CHAPTER XIV

UTILIZATION, DISSIMILATION, AND OXIDATION OF CARBOHYDRATE

THE use of the term "oxidation" to describe the complete breakdown of a foodstuff to CO_2 and H_2O in the tissues carries with it certain traditional physiologic connotations which are no longer acceptable in the light of present-day biochemistry Chief among these is the old conception that the original foodstuff can liberate its energy for use by the tissue by the simple addition of oxygen to its atoms But, as was shown in chapters II, III, and IV, the oxidative breakdown of the energy materials in the tissues is actually a far more complicated matter, involving the processes of oxidoreduction, decarboxylation, addition of CO_2 phosphorylation, hydrolysis and transamination

It is true that the net result of a whole series of reactions may be written as if it were a simple oxidation, as, for example

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

Indeed, it was our limited knowledge of the intermediate steps in this equation which originally led to the inaccurate use of the term "oxidation " But, now that most of the intermediate steps are known the continued use of "oxidation ' for the allover process is a source of great confusion For example, when the biochemist speaks of the "oxidation of lactate," he means specifically the withdrawal of hydrogen from lactate with the formation of pyruvate The physiologist uses the same words to denote the breakdown of lactic acid to CO_2 and H_2O It would be far better for all branches of biological science to use the term "oxidation" in its strict chemical sense, and this is the sense in which it is used in this volume For the complete breakdown of a substrate to CO_2 and H_2O we employ the term "complete oxidation" or "dissimilation" (1)

There is a practical need arising out of the conditions of experimental work for another term, namely, "utilization " In working with the whole living organism or even with isolated tissue in vitro, it is often possible to follow the disappearance of a substrate from the blood or nutritive medium or from the tissues themselves without being able to ascertain the extent to which the oxygen consumed and the CO_2 evolved in the interim were actually concerned with the substrate that disappeared Other substrates are necessarily always present under these conditions and their participation in the reactions under observation is not necessarily ruled out by an approximate equivalence between the respiratory exchange and the dis

appearance of the experimental substrate Such equivalence may be coincidental, for it also happens, not infrequently, that the disappearance of a substrate bears no discernible relationship to the respiratory exchange (2, 3) Under these circumstances when it is impossible to determine the exact chemical fate of the substrate which is disappearing, it is best to employ the term "utilization" As used in this volume, and applied to carbohydrate, for example, it means the disappearance of sugar from the blood or nutritive medium or tissue without storage as glycogen or accumulation as hexose or lactic acid

UTILIZATION OF CARBOHYDRATE AS DETERMINED BY THE DISAPPEARANCE OF THE BLOOD SUGAR IN LIVERLESS ANIMALS

The rapid disappearance of the blood sugar after removal of the liver from the normal animal has been discussed in chapter VII, in connection with the site of formation of the blood sugar The mere withdrawal of sugar from the blood by the extrahepatic tissues cannot, of course, be regarded as proof of its utilization by those tissues However, it has been the universal experience that the carbohydrate content of the tissues and the accumulation of lactic acid or any other substance in the blood do not account for the sugar that disappears from the blood of the liverless animal (4 5 6) The rate of disappearance of blood sugar in such animals may therefore be taken as at least a rough indication of the utilization of sugar by the extrahepatic tissues

In view of this it is significant that the blood sugar disappears after hepatectomy or abdominal evisceration in animals which have been supposed to have ceased utilizing carbohydrate, as judged by the D N ketosis and R Q exhibited before removal of the liver Such evidence is available after hepatectomy of depancreatized birds (7), dogs (8), and rabbits (9) and after evisceration of phlorhizinized dogs (10), of depancreatized and pituitary-diabetic dogs (11), and of normal dogs fasted to the point of so-called "hunger diabetes" (12) A similar incongruity between the conclusions drawn from the classic metabolic criteria and the disappearance of the blood sugar occurs after hypophysectomy of the depancreatized dog (13, 14) and during prolonged injections of epinephrin in the normal dog (15, 16)

UTILIZATION OF CARBOHYDRATE AS DETERMINED BY CHEMICAL BALANCE STUDIES IN LIVERLESS ANIMALS

The groundwork for future chemical balance studies of carbohydrate utilization was laid in the laboratory of H H Dale At that time practical methods for total abdominal evisceration in the cat were not available The liver was left *in situ* with its afferent blood supply tied off However, the asphyxiated organ (with a high free sugar content) could still contribute sugar to the blood by seepage into the vena cava In their later experiments Dale and his co workers (4, 17, 18) recog-

Under the

consumed or from the CO_2 produced If a stable intermediate substance of known chemical composition is formed the R Q may be used to calculate the course of the reaction (20) However it is usually also necessary to determine the amount of original substrate which has disappeared or the amount of intermediate substance

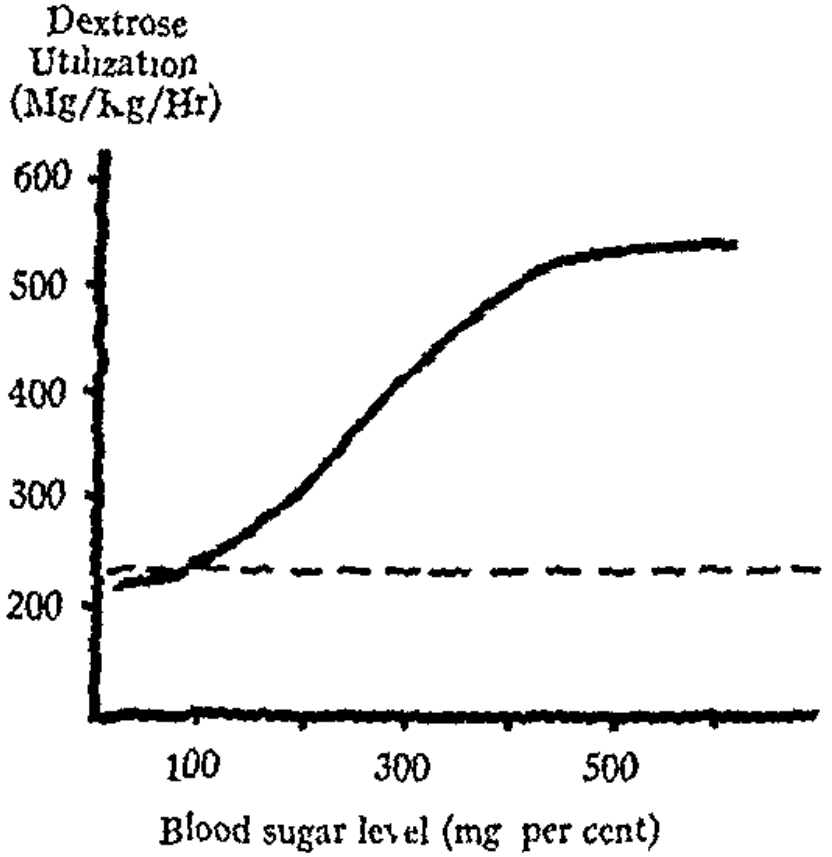

FIG 39 —The relationship between the blood sugar level and sugar utilization in eviscerated normal dogs (Soskin and Levine [5])

which has appeared by chemical analysis When a single substrate is acted upon by an enzyme system and an unknown stable intermediate substance is formed the difference between the theoretical R Q for the complete oxidation of the substrate and the actual R Q obtained may suggest the probable identity of the unknown intermediate (20)

There is no tissue which does not contain a number of substrates and more than one enzyme system In working with a tissue it is therefore desirable to allow it to approach the minimum level of autorespiration (i e to exhaust its own substrates) before the substrate under investigation is added If the R Q of the subsequent

reaction agrees with the chemical determination of the disappearance of the added substrate and the appearance of end products it may then be concluded that the particular enzyme system which it was hoped to engage has operated and that the supposed course of the oxidative process has been confirmed

It is thus apparent that even when one can control the other activities of an isolated tissue and is dealing with a single substrate the R Q is merely confirmatory to the information obtained by chemical analysis When used alone the R Q can at most merely suggest the probable pathway of a reaction which must then be demonstrated by chemical means To illustrate the lack of preciseness of the indications derived from the R Q let us suppose that the substrate is hexose and that no other foodstuff is involved Let us simplify matters further by considering the possible pathways open to just one of its important intermediary metabolites namely pyruvic acid

Table 17 summarizes the rather formidable list of possibilities with the experimental and theoretical R Q of each The various observed total R Q s for pyruvic acid which are cited have been obtained in different tissues and under different circumstances and depend upon the particular combination of the individual reactions favored by the experimental conditions It is obvious that the total R Q of a single tissue like that of the whole body is a composite of many possible R Q s It is also clear that to gain more than the vaguest indication of the fate of the substrate from the R Q alone is a mathematical impossibility Furthermore when the chemical determinations have been made there is little information that the total R Q can add except to act as a check on the possibility that one or more of the end products might have been missed

If we now attempt to apply the foregoing to the interpretation of the R Q *in vivo* there is one further complication which must be mentioned In the body the three main foodstuffs or their breakdown products are constantly available and may be metabolizing simultaneously It has been shown that amino acids may yield the same R Q of unity as is given by carbohydrate (21) Acetoacetic acid if completely oxidized would also yield an R Q of 1 0 In view of the limited significance of the R Q of a single tissue acting on a single substrate what possible meaning can be assigned to the composite R Q derived from many tissues acting on a variety of substrates?

In this predicament the proponents of the R Q have sometimes resorted to the argument that when the R Q of the whole body is determined over a sufficiently long period of time it must represent the resultant of all the R Q s in all the tissues and must therefore ultimately depend upon the chemical composition of the original substrates being oxidized This ignores (*a*) the fact that what constitutes a sufficiently long period of time under various conditions is difficult to determine (in any case practical reasons have usually dictated rather short periods of R Q measurement in the past [22 23 24]) (*b*) the possibility of partial decarboxylation

of some of the intermediary metabolites of the original substrate without further oxidation of the residues, so that the integral of the R Q 's could never equal the theoretical R Q of the original substrate, (*c*) the possibility that some oxygen is used in the formation of storage or excretion products without the formation of equivalent amounts of CO_2, with the same result as in (*b*), and (*d*) the recently discovered mechanism whereby CO_2, hitherto considered to be immediately and

TABLE 17

EXPERIMENTAL AND THEORETICAL R Q 's FOR THE REACTIONS OF PYRUVIC ACID (SOSKIN [49])

REACTION PRODUCTS	MOLES PER MOL. OF PYRUVATE		THEORETICAL R Q	REFERENCES
	O Consumed	CO_2 Produced		
$CH_3 \cdot CHNH_2 \cdot COOH$ (Alanine)	0	0	$\frac{0}{0}=0$	Braunstein and Kritzman (37)
$C_6H_{12}O_6$ (Hexose)	−0 5	0 0	$\frac{0}{-0\ 5}=0$	Benoy and Ell ott (38)
CO_2+H_2O	2 5	3 0	$\frac{3}{2\ 5}=1\ 2$	Long (20)
$COOH \cdot CH_2CH_2 \cdot COOH$ (Succinic acid)	0 75	1 0	$\frac{1\ 0}{0\ 75}=1\ 33$	Elliott and Greig (39) Weil Malherbe (40) Krebs and Johnson (41)
$CH_3 \cdot COCH_2 \cdot COOH$ (Acetoacetic acid)	0 5	1 0	$\frac{1}{0\ 5}=2$	Krebs and Johnson (41, 42)
$CH_3 \cdot COOH+CO_2$ (Acetic acid)	0 5	1 0	$\frac{1}{0\ 5}=2$	Long (20)
$CH_3 \cdot COOH+CH_3 \cdot CHOH \cdot COOH+CO_2$ (Acetic acid) (Lactic acid)	0	0 5	$\frac{0\ 5}{0}=\infty$	Krebs and Johnson (41)

OBSERVED R Q s OF PYRUVATE IN VARIOUS TISSUES

Tissue	Observed R Q	References
Liver	0 82–1 11	Bach and Holmes (43)
Kidney	1 07–1 24	Elliott and Schroeder (44)
Testis	1 17–1 41	Elliott, Greig, and Benoy (45)
Brain	1 18 1 28	Elliott, Greig, and Benoy (45)
Brain	1 28	Long (20)
Liver	1 19–1 76	Elliott Greig, and Benoy (45)

quantitatively excreted, may be held back (temporarily, at least) and its carbon used for the synthesis of metabolic intermediates (25, 26, 27) For example

$$\underset{\text{Pyruvic acid}}{CH_3 \cdot CO \cdot COOH} + CO_2 \rightarrow \underset{\text{Oxalacetic acid}}{COOH \cdot CH_2 \cdot CO \cdot COOH}$$

It is possible that under special experimental conditions, such as prolonged fasting or exclusive high carbohydrate feeding, the R Q does depend largely upon the chemical composition of the original food material which is being dissimilated But even if this possibility be granted, it is perfectly clear that the composite R Q cannot be used to judge the intermediate steps undergone by the foodstuff on its way to complete degradation to CO_2 and H_2O In other words, even if we suppose that the R Q of 0 7 means that the animal is living at the ultimate expense of fat, there is no reason for the further supposition that the fat is being directly and completely oxidized in the extrahepatic tissues (see chap xi) Thus, the R Q has no weight against the previously cited direct chemical evidence that, in its utilization, fat is converted to hexose and ketones by the liver and that these intermediates are dissimilated by the extrahepatic tissues

ATTEMPTS TO VERIFY R Q BY SIMULTANEOUS DETERMINATION OF CARBOHYDRATE UTILIZATION IN INTACT ANIMALS AND IN ISOLATED TISSUES

Despite the inherent limitations of the R Q, a number of investigators have sought direct evidence of its validity as a quantitative index of the type of food stuff that is being dissimilated These attempts have usually consisted of a quantitative comparison of carbohydrate dissimilation as calculated from the R Q, with carbohydrate utilization as determined by chemical balance studies (2, 3, 4, 18 28, 29, 30)

In view of the distinction that we have drawn between dissimilation and utilization, it is evident that they need not tally even if the R Q were a reliable index of complete oxidation, for it would be quite possible for more carbohydrate to be utilized than was dissimilated if some of the carbohydrate were simultaneously being converted into fat or another stable form There is still another difficulty when such comparisons are attempted in intact animals It has been pointed out (chap vii) that the blood sugar level represents a dynamic balance between the rate at which sugar is entering the blood stream from the liver and from any exogenous source and the rate at which it is being removed from the blood by the tissues of the body Thus, a rise in the blood sugar level may result either from an increased rate of sugar supply or from a decreased rate of sugar utilization, or from both together Conversely, a fall in the blood sugar level may be due to decreased supply or increased utilization, or both Nor is it possible to tell which factor is responsible from the mere change in blood sugar level unless one is controlled or eliminated

while the other is observed It is, therefore, futile to attempt to determine the amount of carbohydrate which has been utilized by an intact animal by estimating the difference between its total carbohydrate content at the beginning of an experimental period (plus any sugar which may have been administered) and its total carbohydrate content at the end of the period, for in this procedure the amount of carbohydrate being supplied by the liver is unknown, and any effect of sugar administration on this supply cannot be estimated The experimental conditions are simpler in liverless animals or in isolated tissues, where the available carbohydrate can be estimated or controlled by the investigator

Table 18 summarizes the data of all papers available to the authors from which a comparison of utilization, as determined by chemical balance, and of supposed dissimilation as judged from the R Q , may be attempted A study of the table obviates the necessity for much discussion It is clear that in eviscerated animals and in isolated tissues, as well as in intact animals, there is no correlation between *the results of chemical balance studies and R Q calculations In view of the frequency* and extent of the discrepancies, the few instances in which the results happen to coincide may be regarded as purely fortuitous A somewhat better correlation is obtained in isolated brain tissue than in isolated muscle of the whole living animal This may be ascribed to the fact that the highly specialized nervous tissue does not possess the ability of other tissues for storage and interconversions of foodstuffs and, so far as we know, derives its energy solely from carbohydrate (31, 32, 33, 34) (see chap 1 p 16) However, even under these circumstances, the correlation between chemical balance and the R Q is by no means good This is so even in experiments in which the present authors have improved on the usual technic of chemical balance by a rapid freezing of the control samples (Table 18 no 9)

As was discussed earlier in this chapter, the blood sugar level has an important influence on the utilization of carbohydrate by the living organism Gemmill (3) showed a similar influence of the concentration of sugar in the medium on the carbohydrate utilization of isolated muscle *in vitro* The various data in Table 18 lack a certain amount of comparability because the other investigators failed to take this factor into account Figures 40 and 41 graphically summarize the work of Gemmill (3) and hitherto unpublished data of the present authors for the eviscerated dog isolated muscle, and isolated brain tissue, respectively, in which carbohydrate utilization and R Q calculations are considered in relation to glucose concentration It is apparent that except for isolated brain tissue, there is no concentration of glucose at which carbohydrate utilization and R Q calculations coincide

One must conclude that chemical balance experiments offer neither theoretical nor actual support for the R Q as a measure of dissimilation Since no other validation of the R Q is available at the present time, one must go further and say *that* there is no evidence that the R Q is a measure of dissimilation This leaves us in

TABLE 18

LACK OF CORRESPONDENCE BETWEEN UTILIZATION AND 'OXIDATION"

No	Experimental Conditions	Carbohydrate Utilized*	R Q	Carbohydrate Oxidized †	Percentage of Utilized Carbohydrate Accounted for by 'Oxidation †	Remarks	References
		mg/100 gm		mg/100 gm			(28 29)
1	*Intact mice* a) Glucose injection	67	0 70	0	0	These figures are averages for 38 and 48 animals respectively	
	b) Glucose+insulin	141		128	90		(29)
	Intact rats						
2	a) Fasting+insulin	42	0 74	56	133		
	b) Fasting+epinephrin	12	0 71	0	0		(29)
	Intact rats						
	a) Glucose by mouth	838	0 83	220	91		
3	b) Glucose+insulin	376	0 94	434	116		
	c) Glucose+epinephrin	264	0 83	263	100	' Spinal animals	(4, 18)
		gm		gm			
	Eviscerated cats						
4	a) Glucose+insulin	3 795	1 00	3 595	93		
	b) Glucose+epinephrin	4 340	1 00	2 090	50		(5 48)
		mg/kg		mg/kg			
	Eviscerated dogs (glucose injected intravenously to maintain indicated blood-sugar levels)						
	a) 100 mg per cent	225	0 72	13 4	6	The oxygen consumption + CO_2 production were recorded continuously over a 4 hr period	
	b) 200 mg per cent	315	0 80	94 0	30		
5	c) 350 mg per cent	450	0 89	185 0	41		
	d) 450 mg per cent	520	0 90	280 0	55		
	e) 700 mg per cent	520	1 00	295 0	57		
		mg/100 mg		mg/100 mg			(3)
	Rat diaphragm (in vitro)						
	a) No substrate	0 09	0 73	0 03	33	These data are presented graphically in Fig 41 (p 159)	
	b) Glucose (200 mg per cent)	0 19	0 86	0 20	100		
6	c) Glucose (200 mg per cent)+ insulin	0 34	0 91	0 25	73		
	d) Glucose (500 mg per cent)	0 67	0 86	0 22	30		
	e) Glucose (500 mg per cent)+ insulin	0 81	0 88	0 22	27		
		micro-mol/gm		micro-mol/gm			(1)
	Pigeon skeletal muscle (in vitro)						
7	No substrate	11 2	0 97	29 7	265	Calculated averages from Table VIII of the paper by Stadie and Zapp	
	Cat skeletal muscle (in vitro)						
	No substrate	−4 3	1 00	9 7	Not calculable		(46)
		mg/gm		mg/gm		Calculated from the data in Table II of the paper by Elliott et al	
	Rat-brain homogenate (in vitro)						
	a) No substrate	0 58	0 87	1 34	231		
8	b) Glucose	3 59	0 91	2 80	78		
	c) Glucose+insulin	3 73	0 93	3 29	88		(47)
	Rat-brain homogenate (in vitro)					Better correspondence between 'oxidation and utilization was obtained when the control samples were frozen (dry ice) as soon as the brain was removed from the animal Experiments (b) and (c) were done in this manner	
	a) No substrate		0 81	0 95	2 78	343	
9	b) No substrate (cf under Remarks)	3 60		1 00	5 73	104	
	c) Glucose	6 27		1 02	5 58	89	

* Utilized = change in total carbohydrate during experimental period.

† Oxidized = amount of carbohydrate dissimulated as calculated from the oxygen consumption and the R Q

continues high for some time after intestinal absorption is complete or the injection has ceased The total increment in the oxygen consumed (and the corresponding extra energy expenditure) is known as the "specific dynamic action' (S D A) of the foodstuff given The magnitude of the S D A differs for the different foodstuffs For carbohydrate it *approximates* 10 per cent of the caloric value of the amount of sugar administered

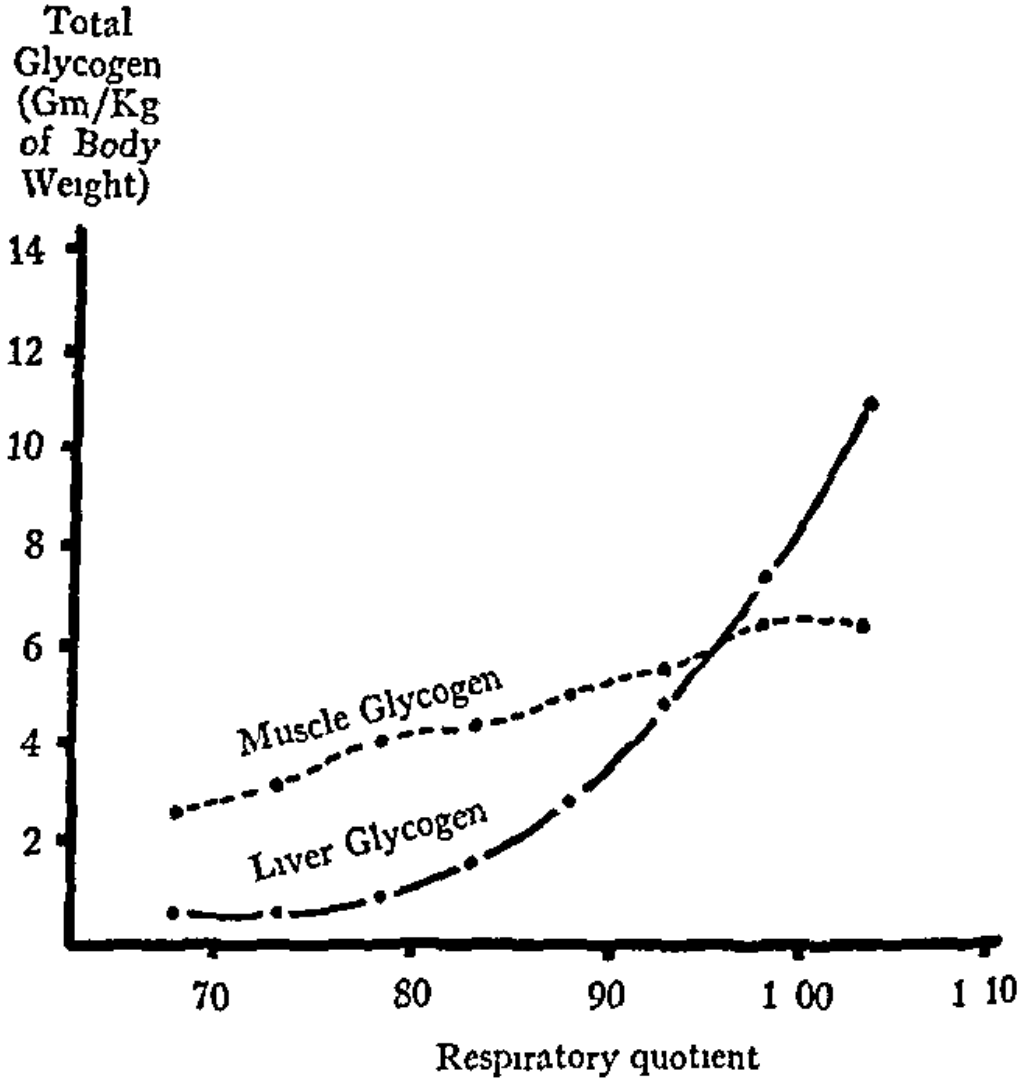

Fig 41a —Relationship between muscle glycogen liver glycogen and R Q (Bridge [36])

Various explanations of the S D A have been advanced (22). The mechanism is undoubtedly different for each of the foodstuffs The work of Wierzuchowski (35) *is the most illuminating as regards carbohydrate He injected* glucose intravenously into dogs at rates ranging from 1 to 9 gm per kilogram per hour and observed the heat production, the R Q , and the sugar and lactic acid levels of blood and urine He then correlated the S D A with his other data at all rates of glucose injection and found that there was a good proportionality between the S D A and the amount of glucose "assimilated ' (the amount of glucose injected minus the

amount excreted in the urine) The glucose equivalent of the oxygen consumed was not clearly related to the S D A , neither was the fat formation, as judged by the slight rise of the R Q above unity and other criteria He therefore concluded that the S D A was related to the amount of glucose stored, which for practical purposes means the amount of glycogen formed

Simultaneously with the increased oxygen consumption following carbohydrate intake there is an even greater rise in CO_2 production, so that the R Q is elevated (chap xi) Bridge (36) has pointed out a relationship between the rise in R Q and glycogen deposition similar to that found by Wierzuchowski for the S D A Figure 41a, taken from Bridge shows the correlation between the R Q and the glycogen contents of liver and muscle in a series of rabbits at various intervals after carbohydrate administration It will be noted that the curve relating the R Q to liver glycogen is remarkably smooth

The work of Wierzuchowski and of Bridge suggests that the S D A or the R Q , or both, could be used as an index of glycogen formation in the intact animal or in man when the sampling of tissues is impossible or undesirable There is a good theoretical basis for this application, quantitatively as well as qualitatively We have seen in chapter iv (see Fig 20) that the synthesis of glycogen requires energy which is derived from oxidative steps in the breakdown of glucose From *in vitro* experiments it can be calculated that the oxidation of 1 mol of glucose provides the energy for the phosphorylative synthesis of 6–12 mol of glycogen From this one might predict that the S D A of glucose would lie between 8 and 17 per cent of the amount of glucose retained The observed S D A of 10 per cent is well within this theoretical range It remains for future work to compare the S D A and the R Q with chemical determinations of glycogen deposition under conditions which would be feasible for clinical use

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PART IV

THE ROLE OF THE ENDOCRINE GLANDS IN CARBOHYDRATE METABOLISM

level of 45 mg per cent within 5 hours One milligram of pure crystalline insulin contains 22 such units

From a historical standpoint and because of its importance as a research tool and as a therapeutic agent, insulin may be regarded as the dominant instrument in the symphony of endocrine action that results in normal carbohydrate metabolism It should be remembered that any particular hormone is merely one of the components of the endocrine balance and that its actions depend upon the presence and simultaneous influences of the other hormones In this sense it is difficult to deal with one hormone at a time But, since it is even more difficult to describe the complicated actions and interactions of all the endocrine glands in a parallel fashion it does serve a useful purpose to discuss the subject as if insulin were carrying the leitmotiv of the symphonic work while the other endocrine instruments amplified or modified the theme

THE REGULATION OF INSULIN SECRETION

In the post absorptive state and in the absence of physical emergencies or emotional crises the pancreas probably secretes small amounts of insulin into the blood continuously This constant secretion is a prerequisite for the efficient functioning of the hepatic regulating mechanism, which is the most important factor in the maintenance of the normal blood sugar level (9, 10) (cf p 248) Hédon (11) has shown that a deficiency of insulin and a consequent rise in the blood sugar level begins immediately after removal of the pancreas Soskin and his co-workers (12) found that it required a constant injection of insulin to maintain a constant normal blood sugar level in depancreatized dogs The latter investigators further showed that *no extra secretion of insulin was necessary* for an adequate disposition of a sudden influx of carbohydrate (cf chap xxi, p 249) However, this does not contradict the considerable body of evidence which indicates that *extra insulin is ordinarily secreted* as a result of hyperglycemia following carbohydrate intake (13, 14) or as a consequence of central nervous system activity transmitted through the right vagus nerve (15 16, 17)

It has been shown that under special experimental conditions hyperglycemia may stimulate the pancreas both directly and by way of the nervous system (18 19) In the normal intact animal these mechanisms for counteracting hyperglycemia contend with other mechanisms that tend to raise the blood sugar level For example, asphyxia and certain drugs like metrazol, ordinarily result in hyperglycemia In animals in which the adrenal medullae have been destroyed, these same agents cause hypoglycemia (20, 21) But when the right vagus nerve is cut in an adrenal medullectomized animal, the hyperglycemic agents produce no effect on the blood sugar level (20 21) It is evident that vagal stimulation of extra insulin secretion acts as a restraining counterregulation in limiting the hyperglycemic effects of the adrenal medulla and the sympathetic nervous system The

adrenal medulla and the sympathetic nervous system, on the other hand, may be regarded as emergency safeguards against hypoglycemia that is too rapid or too severe to be adequately handled by other mechanisms

It is beyond the scope of this volume to discuss these emergency mechanisms in detail It may be pointed out, however, that their peculiar status is revealed by the fact that adequate regulation of the blood sugar level (except for an increased sensitivity to insulin) ordinarily persists even after all possible influence of the nervous system has been eliminated This has been shown after denervation of the liver (22), denervation or grafting of the pancreas (23, 24, 25, 26, 27, 28, 29, 30), denervation or destruction of the adrenal medulla (31, 32, 33), bilateral vagotomy (34, 35), and total sympathectomy (32, 36)

THE KNOWN PHYSIOLOGICAL EFFECTS OF INSULIN

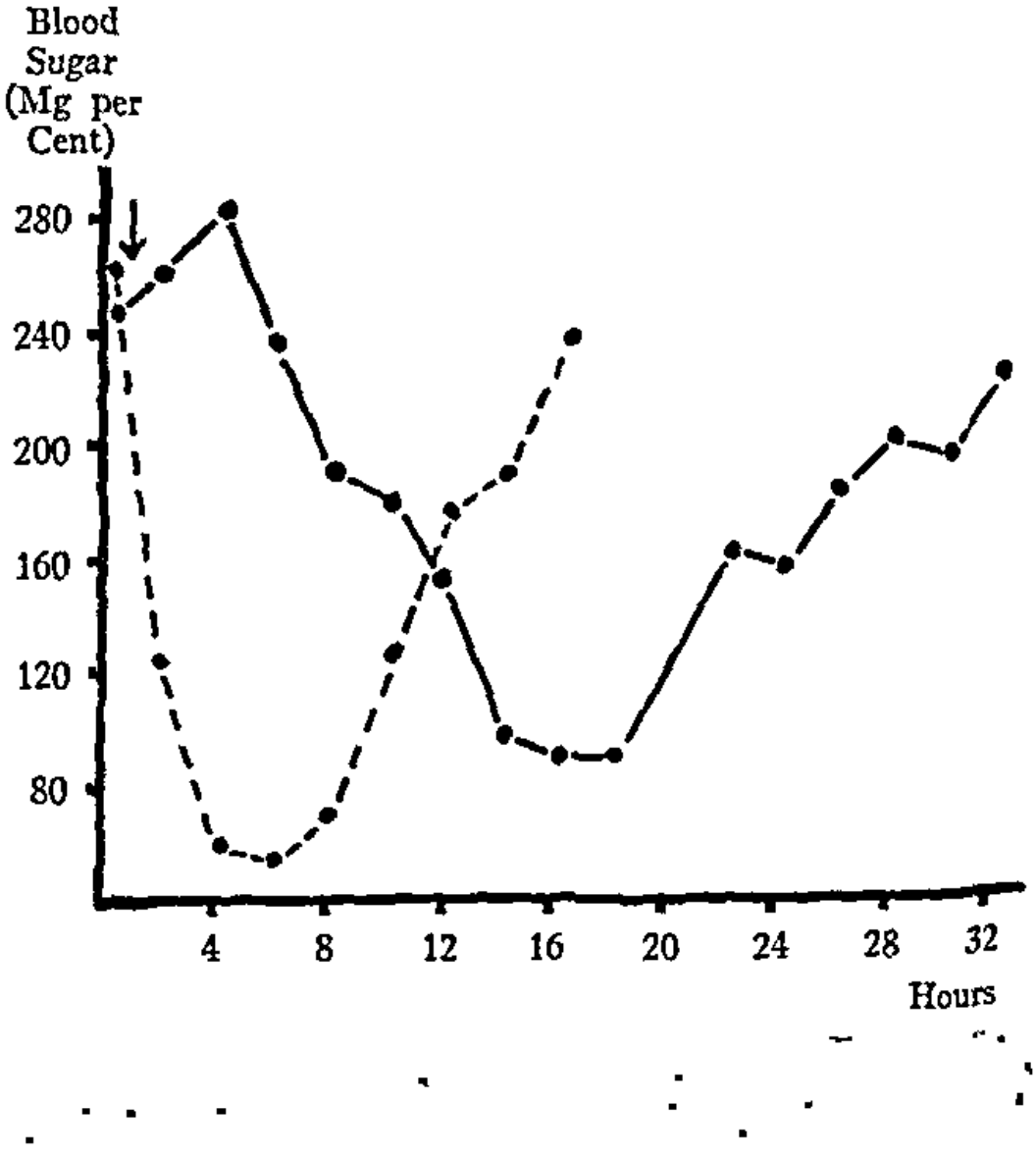
1 *Hypoglycemia* —Since highly purified insulin has been available for experimental and clinical use, it has been administered to animals and humans under the most diverse conditions Except for differences in the magnitude of the effect obtained with a given amount of insulin (so-called "sensitivity'), a hypoglycemic effect is invariably obtained, regardless of the state of the animal This is true for animals at any age in whatever state of nutrition, and lacking the various endocrine glands or visceral organs (37, 38, 39 40) It is clear, therefore, that the hypoglycemic effect of insulin is a general one, which is not mediated by any particular organ or tissue Figure 42 shows the typical curves of action of regular and of protamine insulin

Numerous attempts have been made to determine whether the action of insulin might be on the blood itself It has been impossible to demonstrate any change in blood *in vitro* by the addition of insulin (41, 42) At one time it was claimed that insulin changed the blood glucose to a more reactive form (43, 44) (γ glucose), but this was never substantiated (45, 46) It is also known that insulin has no influence on the distribution of glucose between plasma and red blood cells (47) or on the rate of glycolysis of the blood sugar (48, 49) It seems certain, therefore, that the lowering of the blood sugar level *in vivo* under the influence of insulin is a result of the more rapid withdrawal of sugar from the blood by the other tissues A decreased supply of sugar to the blood from the liver is an additive factor (50, 51, 52)

2 *Glycogen deposition* —Next to its hypoglycemic effect, the glycogenetic effect of insulin in skeletal muscle is its most thoroughly substantiated direct action It is readily demonstrable *in vitro* on thin sheets of muscle (diaphragm or abdominal muscle of the young rat) in the Warburg apparatus (53, 54, 55) It is important to remember, however, that this action of insulin *in vivo* is related to the existing blood sugar level from moment to moment both because of the amount of sugar available for deposition and because of the secondary counterregulations evoked

by hypoglycemia Thus, unless the blood sugar is maintained by the administration of sugar, the hypoglycemia resulting from insulin action will evoke a secretion of epinephrin from the adrenal medulla, which, in turn, may mask the glycogenetic effect of the insulin by causing a rapid breakdown of muscle glycogen to lactic acid

That insulin influences the deposition of liver glycogen is evident from the characteristically low glycogen levels of the diabetic liver (56, 57) and their return to



normal with insulin treatment (58, 59) But there is a paradoxical situation as regards the effects of administered insulin in normal animals, for (with a single unexplained exception [60, 61]) all normal animals invariably exhibit a decreased amount of hepatic glycogen after insulin administration (62, 63, 64) Part of this effect may be ascribed to the hypoglycemia induced secretion of epinephrin and

the consequent breakdown of liver glycogen to blood sugar But this is by no means the whole explanation for Bridge (65) has shown that insulin administered with sufficient glucose to maintain a certain blood sugar level results in a smaller deposi tion of hepatic glycogen than the administration of that amount of sugar alone which will reproduce the same blood sugar level He also showed that this anom alous effect of insulin in normal animals could be obtained in the absence of the adrenal medulla

The normal heart like skeletal muscle, deposits increased glycogen under the influence of insulin (66 67) But cardiac glycogen is apparently more dependent upon the concentration of sugar available in the blood than is the glycogen of other organs for the heart of the completely depancreatized animal may contain large amounts of it (68 69 70)—amounts which are reduced by restoring the blood sugar level to normal with insulin The finding of Junkersdorf (71) of a high glycogen content in the cardiac muscle of phlorhizinized dogs with low blood sugar levels also suggests the possibility of the formation of cardiac glycogen *in situ* from non carbohydrate sources

The glycogen content of the brain and nervous tissues on the other hand is in fluenced little if at all by either the blood sugar level or by the insulin content of the blood (72 73) Indeed it seems likely that the small amount of glycogen which is found in these tissues has more structural than metabolic significance, since the amount is little affected by various nutritional physiological and phar macological factors (74 75)

3 *Antiketogenesis* —As outlined in detail in chapter x, ketogenesis in the liver is best correlated with a lack of glycogen Accordingly insulin is antiketogenic (76 77, 78) under conditions in which it increases liver glycogen (in the diabetic organism), but it may actually be ketogenic (79 80) under conditions in which it decreases liver glycogen (in the non-diabetic organism) Insulin has no influence whatever on the rate of disposal of ketone bodies by the extrahepatic tissues (81 82)

4 *Change in the R Q* —Whatever the significance of the R Q (chap xiv), insu lin has a definite effect upon it But the situation with respect to the difference be tween the normal and the diabetic organism and the influence of the amount of car bohydrate available is somewhat similar to that which obtains for glycogen depo sition in the liver Thus in the absence of insulin the diabetic organism fails to ex hibit the rise in the R Q which follows the administration of sugar to the normal animal (83 84) The administration of insulin alone to the fasting diabetic organ ism results in an elevation of the quotient (85 86) However, insulin administra tion to the fasting normal organism results in variable changes of small magnitude (87 88 89), although insulin plus sugar does cause a more abrupt and more pro-nounced rise in the R Q than does sugar alone Insulin has either no effect on the oxygen consumption or may actually decrease it (54, 55, 67, 90)

When insulin does affect the R Q , the results bear no quantitative relation to the fall in the blood sugar level According to Bridge (91), the R Q changes correlate best with the level of hepatic glycogen (see chap xiv, p 161)

5 *Decrease in serum inorganic phosphate* —In the absence of insulin the diabetic organism exhibits an abnormally high level of inorganic phosphate in the blood (92, 93) This is corrected by treatment with insulin (93, 94) The administration of insulin to the normal animal causes a diminution of serum inorganic phosphate below the normal level (95, 96, 97) There have been variable and contradictory reports concerning supposedly parallel changes in the hexosemonophosphate content of muscle, presumably due to the entrance of the blood serum inorganic phosphate into muscle in this esterified form (98, 99) But Soskin and

TABLE 19

CHANGE IN INORGANIC PHOSPHATE (P_i) AND TOTAL ACID-SOLUBLE PHOSPHATE (P_T) OF THE BLOOD AND IN HEXOSEMONOPHOSPHATE (HmP) OF THE MUSCLE (SOSKIN *et al* [42])

(In Milligrams per Cent)

Experimental Conditions	Dog No	Change in Blood		Change in Muscle HmP*
		P_i	P_T	
Depancreatized dogs given epinephrin (0 1 mg/kg subcutaneously)	1	−0 3	0	+ 9 5
	2	−0 1	+3 0	+10 9
	3	−0 4	0	+ 9 4
Adrenalectomized dogs given insulin (0 3 unit/kg subcutaneously)	1	−1 9	0	− 0 5
	2	−1 6	+2 0	− 0 3
	3	−1 2	+3 0	+ 0 3

* In terms of phosphate

his co-workers (42) have shown that the phosphate changes in blood and muscle are not directly related to each other and that only the fall in the blood inorganic phosphate is a direct consequence of insulin action The confusion was due to the counterregulatory reactions, whereby excessive insulin activity evokes a secretion of epinephrin, and vice versa When the actions of the individual hormones are isolated by excision of the counterregulating gland, the unopposed action of the administered hormone can be observed (Tables 19 and 20)

The administration of insulin to the normal intact animal is followed by both the blood and the muscle phosphate effects In the absence of the adrenal glands, the action of insulin on the blood phosphate persists, while the hexosemonophosphate in muscle is not affected The responsibility of reflexly secreted epinephrin for the muscle phosphate changes after insulin administration also accounts for the absence of those changes in normal animals when sufficient dextrose to prevent hypoglycemia is administered with the insulin Conversely, epinephrin in the nor-

mal animal causes both a fall in the inorganic phosphate in the blood and a rise in the hexosemonophosphate in the muscle But in the depancreatized animal, only the muscle effect of epinephrin occurs

The action of insulin in lowering the blood inorganic phosphate is not explained by a loss of phosphate from the blood, for the total blood phosphate remains unchanged It seems probable, therefore, that there is an esterification of the inorganic phosphate within the blood (42, 100), although the nature of the phosphate compound which is formed is, as yet, unknown

6 *Decrease in serum potassium* —A number of investigators have observed a lowering of the potassium content of the blood serum following the administration of insulin to normal animals (101, 102, 103) There has been no elucidation of the

TABLE 20

CHANGE IN BLOOD INORGANIC PHOSPHATE (P_i) AND IN TOTAL ACID SOLUBLE PHOSPHATE (P_T) (SOSKIN *et al* [42])

(In Milligrams per Cent)

The maximum decrease in blood inorganic phosphate (P_i) obtained with glucose in any depancreatized animal was 0 4 mg per cent. Hence no change in P_i of this amount or less was considered to be significant throughout our work

Type of Animal	Glucose					Insulin					Epinephrin				
	No of Dogs	Decrease in P_i			Av Rise in P_T	No of Dogs	Decrease in P_i			Av Rise in P_T	No of Dogs	Decrease in P_i			Av Rise in P_T
		Min	Max	Av			Min	Max	Av			Min	Max	Av	
Normal	5	0 5	1 2	0 8	2 0	20	0 7	2 0	1 2	2 0	9	0 4	1 7	1 1	2 0
Depancreatized	7	0	0 4	0 2	1 0	9	1 3	2 8	1 9	1 0	14	0 6	0	0 3	3 0
Adrenalectomized	3	0 7	2 8	1 6	6 0	7	0 5	2 0	1 2	3 0	3	1 1	1 2	1 2	3 0

mechanism of this effect, except perhaps in so far as it may be related to the increased rate of entry of sugar into tissues under the influence of the hormone Fenn has shown that potassium enters tissues in proportion to the amount of carbohydrate which is taken up (104)

7 *Influence on nitrogen metabolism* —In the absence of insulin the diabetic organism excretes abnormally large amounts of nitrogen in the urine (105, 106, 107) This indicates that insulin must act to inhibit protein catabolism at some point The *in vitro* work of Bach and Holmes (108) with liver slices showed that insulin inhibits the deamination of amino acids, as judged by the decreased rate of appearance of [illegible]

similar experiments, were able to confirm this insulin effect with *d* alanine but not

with the naturally occurring *l* alanine as had Bach and Holmes This n trogen sparing effect of insulin was further demonstrated by Gaebler and others (*110 111*) in an indirect way They found that whereas extracts of the anterior pituitary ad ministered to normal animals resulted in nitrogen retention the same treatment in diabetic animals caused an increased nitrogen excretion

The administration of insulin to the normal animal is followed by uncertain and contradictory results (112 113) There may be either no change or an actual in crease in nitrogen excretion However the amino acid level of the blood does de crease s gnificantly (114 115) Like other effects of insulin under similar circum stances this is probably due to the counterregulatory effects of other glands par ticularly the adrenal medulla Luck and his co workers (116) have shown that in adrenal demedullated animals insulin fails to lower the blood amino acids while epinephrin will do so just as it does in the normal animal It seems reasonable to conclude therefore that the apparent influence of insulin on the amino acid level of the blood of the normal animal is actually due to the reflex secretion of epi nephrin resulting from hypoglycemia This same sequence of events could of course also account for the increased excretion of nitrogen which sometimes fol lows the administration of insulin to normal animals for epinephrin has been shown to increase protein catabolism

However it is not at all certain that as de from secondary effects due to epi nephrin secretion insulin does not have a direct action of its own upon the blood amino acids Mirsky and his co workers (117 118) found that in eviscerated and nephrectomized dogs maintained by a constant injection of insulin and glucose the blood amino acids rose more slowly and injected glycine disappeared more rapidly than in similar animals maintained on sugar alone Since the absence of the liver and kidneys precludes a loss of the amino acids by deamination these experiments suggest that insulin facilitates the use of amino acids in the muscles for synthetic purposes either directly or indirectly (see chap xix p 235)

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CHAPTER XVI

THE MODE OF ACTION OF INSULIN

A MORE detailed examination of the physiological effects of insulin sheds some light on the manner in which insulin influences carbohydrate metabolism It may be well to begin by directing our attention to skeletal muscle, because this tissue comprises about 50 per cent of the body weight, because it is a less complicated organ, in a biochemical sense, than is the liver, and because more data concerning it are available

INSULIN AND GLYCOGENESIS IN SKELETAL MUSCLE

Although it is facilitated by insulin, the deposition of glycogen can occur in the complete absence of the hormone (1, 2) The fact that insulin is not essential for glycogen formation has received *in vitro* confirmation from the work of Cori and his co-workers (3, 4) They synthesized glycogen from glucose in the test tube in the presence of the necessary enzymes but without insulin Indeed, they were unable to demonstrate any effect when insulin was added to their system (5 6) In the living animal, Dambrosi (7) and Lukens (8) have shown that the absence of insulin does not even limit the extent to which glycogen is restored after its depletion by exercise It is the rate of restoration of glycogen which is deficient, for, whereas in the normal animal it took 1 hour to restore the pre-existing glycogen level, the muscle glycogen of the completely depancreatized animal was restored just as fully in 4 hours Insulin, therefore, exerts its influence on the rate of glycogen formation

The major factor, other than insulin which determines the rate of glycogen synthesis is the concentration of sugar present This is, of course, in accord with the general nature of all enzyme reactions Cori *et al* (9) have shown that the amount of glycogen deposited in the liver of a given experimental animal depends upon the height at which the blood sugar level is maintained rather than upon the total amount of sugar given It has been possible in our own laboratory (10) to demonstrate this relationship for muscle even more clearly on rat diaphragm *in vitro* by the Warburg technic Figure 43 shows the increasing amounts of glycogen deposited at increasing sugar concentrations, with or without added insulin It will be noted that at the highest concentrations of sugar the insulin exerted no significant effect over and above the effect of sugar concentration This relationship of insulin action to sugar concentration is consistent with other actions, which are to

be discussed later In other words, insulin enables the tissues to do at low or physiological sugar concentrations that for which they would otherwise require very high sugar concentrations

INSULIN AND THE UTILIZATION OF SUGAR BY SKELETAL MUSCLE

One of the most firmly intrenched notions about insulin in the metabolic literature is that it increases the dissimilation of carbohydrate This is without basis in fact, for, as pointed out in chapter xiv, no over all measure of dissimilation in the living organism is yet available The supposed proof for the mistaken asser

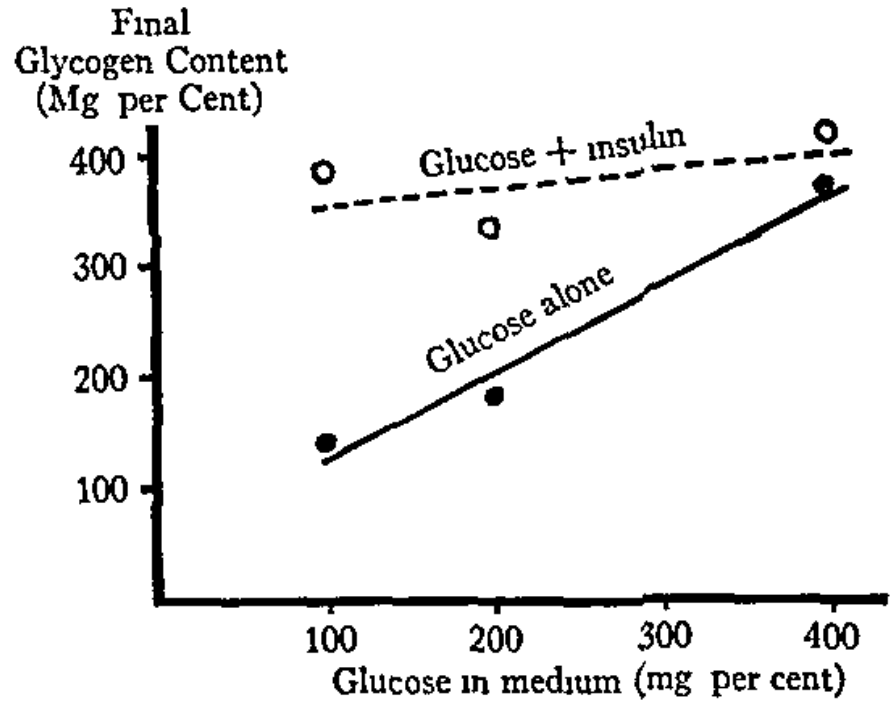

FIG 43 —Influence of sugar concentration on deposition of glycogen in rat diaphragm *in vitro* with and without insulin (Hechter *et al* [10])

tions is based upon calculations of so-called oxidation" from the R Q (see chap xi) and the estimation of utilization from carbohydrate balance experiments

Wierzuchowski (11) used R Q measurements to calculate the amounts of sugar oxidized before and after the administration of insulin in two normal unanesthetized dogs receiving constant intravenous injections of glucose (Table 21) According to these calculations one of the animals "oxidized ' 21 5 per cent of the assimilated sugar before insulin administration and 27 3 per cent after insulin But the other animal "oxidized ' 19 1 per cent before insulin and 19 0 per cent after insulin The results from the two dogs were averaged, and the conclusion arrived at was that insulin had increased the oxidation of assimilated glucose from 20 3 per cent to 23 2 per cent!

Best, Dale, Hoet, and Marks (14) measured oxygen consumption and made carbohydrate balance studies on the same eviscerated spinal cats In the absence of the liver they found that an increased amount of sugar disappeared from the blood following insulin administration and that *the sugar which disappeared was* equal to the sum of the glycogen deposited in the muscles and the glucose equiva lent of the oxygen consumed In accordance with the state of knowledge at that time, Best *et al* (15) concluded that the effects of insulin in excess represent an in tensification of its physiological effects, *including the acceleration of the combus*tion of carbohydrate Hence their work has since been quoted as proof that insulin increases the dissimilation of carbohydrate

A re examination of their original data shows that this conclusion was not war ranted Table 24 summarizes the pertinent figures from the experiments which they themselves selected as being most free from technical criticism The right

TABLE 24

INFLUENCE OF INSULIN ON GLUCOSE OXIDATION OF EVISCERATED SPINAL CATS (BEST *et al* [14])

ORIGINAL DATA					RECALCULA TION
Exper ment No	Insul n (Units)	We ght of Cat (Kg)	Duration of Experiment (Min)	Glucose Ox d zed (Mg)	Glucose Ox d zed (Mg/Kg/Hr)
5*A*	0	3 2	50	1 045	392
5*B*	20	3 2	150	2 970	371
6	30	2 6	210	2 595	285
7	25	2 8	250	3 079	264

hand column is our own recalculation of the amounts of sugar "oxidized ' in milli *grams per kilogram per hour, inserted in order to make these values comparable* It may be seen that animal No 5 'oxidized" less sugar after insulin than before Animals Nos 6 and 7, for which no pre insulin periods are given, "oxidized less sugar after insulin than animal No 5 "oxidized ' without insulin It is clear that this work offers no support for the contention that insulin increases the rate either of utilization or of the so-called "oxidation of carbohydrate

The more recent work of Soskin and his co workers (16, 17) has confirmed the fact that insulin does not increase the utilization of carbohydrate in the organism as a whole, while at the same time giving some insight into the reasons for the previous confusion The form of the experiments was a chemical balance study in liverless dogs, as described in chapter XIV, where the relation of carbohydrate utilization to blood sugar level was discussed Experiments similar to those which were done on the normal animals, were repeated on completely depancreatized dogs which had been deprived of food and insulin for 3 days Figure 44 summarizes

the results and compares them to those obtained in normal animals It may be seen that dextrose utilization in the depancreatized dog is qualitatively similar to that in the normal dog In both types of animal the rate of utilization depends upon the height of the blood sugar level Within a wide range of blood sugar values the diabetic dog utilizes less sugar than does the normal dog at any particular glycemic level But, above certain high levels this difference disappears and both types of animal use the same amounts of carbohydrate at the same blood sugar

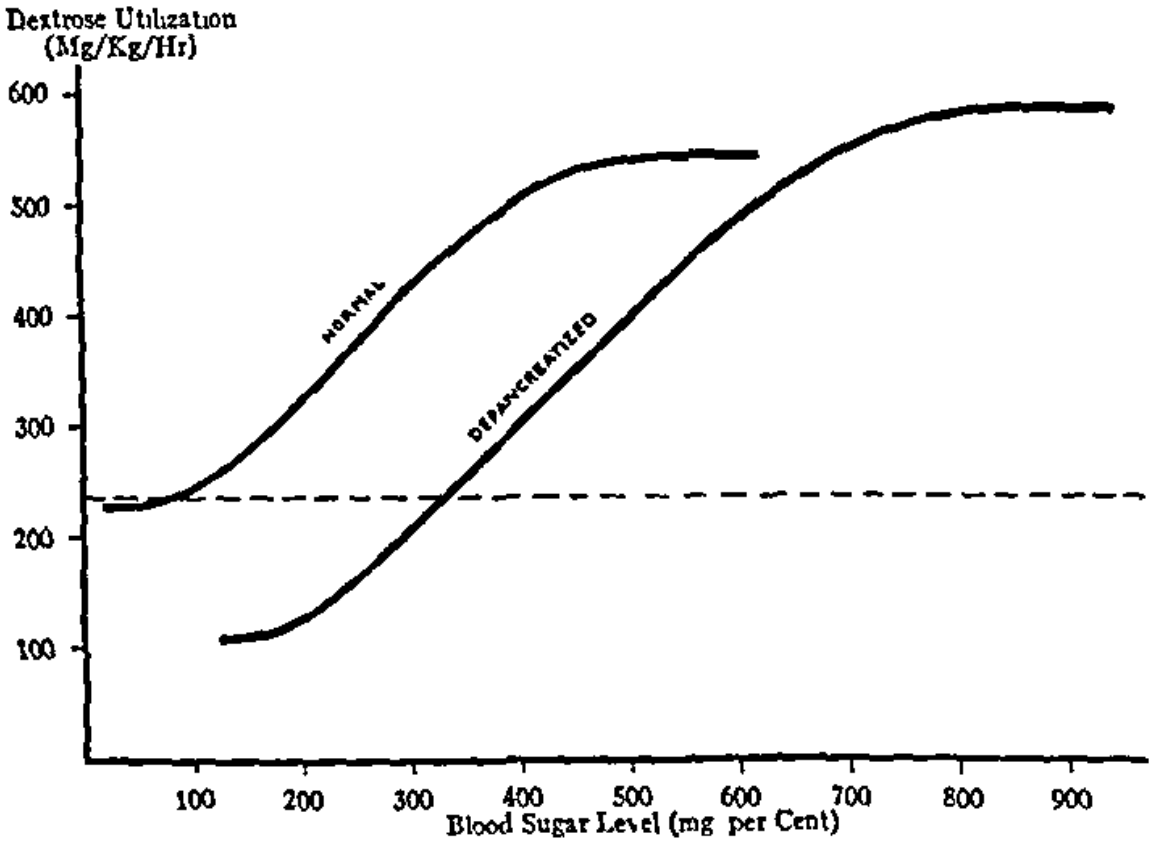

FIG 44 —Relationship between blood sugar level and dextrose utilization in normal and in depancreatized dogs (Soskin and Levine [16])

levels *When, however, one compares the rate of utilization of the normal animal at its usual normal blood sugar level with the rate of utilization of the diabetic animal at the hyperglycemic levels which it ordinarily maintains, it is apparent that the diabetic animal habitually uses as much or more sugar than the normal animal*

It is also clear that, when one administers insulin to a diabetic animal, two mutually counterbalancing effects are obtained there is a potential increase of the amount of carbohydrate that can be utilized at the pre-existing blood sugar level, but there is also a coincident reduction in the level The net result is no change in the rate of utilization In view of these results, insulin cannot be regarded as essential to the utilization of dextrose or even as a determining factor, so far as the

net result is concerned It apparently plays the part of a catalyst or activator in a process which can proceed at a slower rate in its absence More specifically, it permits rates of carbohydrate utilization at low blood sugar levels which in its absence would require abnormally high blood sugar levels

The question then arose as to whether the amounts of insulin available in the normal animal were such as to result in maximal rates of utilization at any given blood sugar level To answer this question, carbohydrate balance experiments were performed on eviscerated normal animals maintained at particular blood sugar levels despite the constant administration of large amounts of insulin (17)

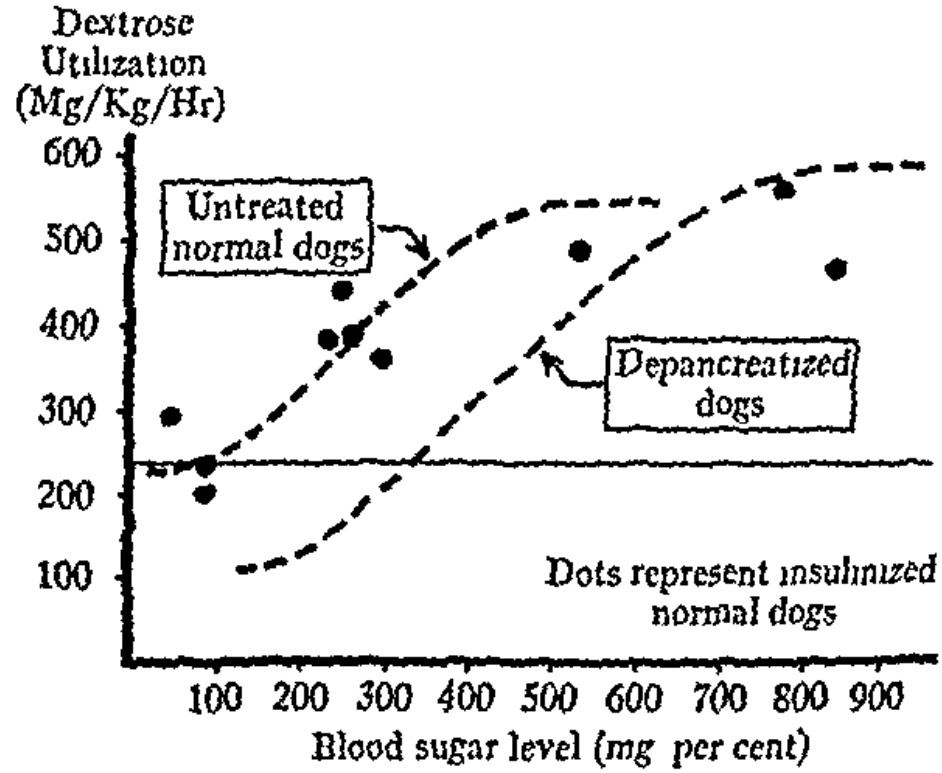

FIG 45.—*Influence of administered insulin on sugar utilization in normal dogs (Soskin and Levine [17])*

normal animal is already optimal as regards the utilization of sugar, so that additional insulin causes no change But this is not the case as regards the storage of muscle glycogen, which is increased as a result of additional insulin

Considering the fact that the lack of insulin causes a diminution in both utilization and storage of carbohydrate by the peripheral tissues at any given blood sugar level, it seems probable that insulin acts by promoting the conversion of glucose into some intermediate substance which is necessary for both processes It may be supposed that the rate of formation of the intermediate substance depends upon

the concentration of the blood sugar and upon the amount of insulin present In the untreated depancreatized animal the increased concentration of blood sugar can by itself lead to the formation of sufficient intermediate substance to support the normal rate of catabolism However, there is little or no excess of the intermediate substance available for synthesis to glycogen The administration of insulin to the depancreatized animal increases the amount of intermediate substance formed at any blood sugar level The animal now resembles the normal in having available sufficient intermediate substance to maintain the normal or maximal rate of catabolism even at normal blood sugar levels There is now also available additional intermediate substance for synthetic purposes In the normal animal, in

TABLE 25

INFLUENCE OF INSULIN, IN RELATION TO THE BLOOD-SUGAR LEVEL, ON THE RATE OF ENTRY OF GLUCOSE INTO THE PERIPHERAL TISSUES OF LIVERLESS ANIMALS

Blood-Sugar Level Maintained (Mg/100 Cc)	Milligrams of Glucose Entering the Tissues per Kilogram of Body Weight per Hour			Reference
	Depancreatized	Normal	Normal + Added Insulin	
45		28	221	Soskin and Levine (16, 17)
80		104	361	
160	79	125		
230	50	262	340	
525	388		577	
620	415	400	578	
750	471		491	
200		124	406	Best *et al* (14 15)
240		150		
325		200	1 008	

which sufficient intermediate substance is already present to allow the catabolic reactions to proceed at their maximal rate, additional intermediate substance resulting from insulin administration is reflected only in increased glycogen synthesis

If the action of insulin in the tissues is to promote the conversion of glucose into some intermediate substance which is necessary for both utilization and glycogenesis, a consistent effect of the hormone should be an increased rate of entry of sugar into the tissues, regardless of the fate of the sugar thereafter Ample data to show that this is the case were furnished by the carbohydrate balance experiments, in which sugar was constantly injected in order to maintain constant blood-sugar levels (16, 17) Table 25 summarizes these data, as well as the results of comparable experiments of Best *et al* (14, 15)

INSULIN AND THE DISSIMILATION OF CARBOHYDRATE BY SKELETAL MUSCLE *in vitro*

Since the advent of the Warburg technic, there have been a number of attempts to demonstrate the action of insulin *in vitro* These attempts have been successful as regards the deposition of glycogen in isolated muscle (p 169) but have been uniformly unsuccessful in showing any influence of insulin on so called 'oxidation or dissimilation of carbohydrate in *mammalian muscle* (*18 19 20*) *As in the whole* animal, insulin causes either no change or an actual decrease in oxygen consumption, and there is no correlation between the oxygen consumed and the sugar which disappears (Table 18, p 157)

In contradistinction to the results obtained in mammalian muscle, Krebs and Eggleton and others (21, 22) were able to demonstrate an increased oxygen consumption under the influence of insulin in minced pigeon breast muscle These experiments were performed in the presence of glucose as the substrate and with the addition of citric acid as a catalytic agent The high R Q obtained under these circumstances led to the conclusion that the increased oxygen consumption resulting from the addition of insulin signified a stimulation of carbohydrate "oxidation' by the hormone Using the same tissue and pyruvate as the substrate, Rice and Evans (23) demonstrated an increased oxygen consumption with a coincidentally increased disappearance of pyruvate under the influence of insulin Apparently, an insulin effect on some oxidative process is obtainable in pigeon breast muscle

The work on the muscle of birds tends to confuse the picture of insulin function rather than to clarify it for it must be pointed out that, of all the experimental animals pigeons are about the least suitable from which to draw conclusions of general significance It takes relatively enormous doses of insulin in the intact bird to *produce even a small fall in the blood-sugar level On the other hand, the removal*

of the fate of pyruvate in mammalian muscle *According to Hock and Bollman* (26), administered pyruvate is disposed of just as rapidly by the completely depancreatized dog as by the normal dog, while Bueding and Himwich (27) have shown that the administration of insulin with glucose actually results in a greater rise of pyruvic acid in the blood than does the injection of the carbohydrate alone In view of these facts, it seems necessary to reserve the work on pigeon breast muscle for future interpretation, for it is impossible to correlate or reconcile the results in birds with the much larger body of information obtained from mammals

THE INFLUENCE OF INSULIN ON THE LIVER

Evidence as to the mode of action of insulin in the liver is less abundant than the evidence for muscle, chiefly because hepatic tissue appears to be so sensitive to environmental factors that relatively few successful *in vitro* or *perfusion* experi

ments have been reported When studying the intact living organism, the results are difficult to interpret because of the many regulatory and counterregulatory influences to which the liver is subject Nevertheless, there are sufficient data to show that the actions of insulin on the liver are correlated with the blood sugar level, as they are in muscle

Issekutz and Szende (28) were the first to demonstrate that insulin inhibits hepatic glycogenolysis They showed that livers removed from frogs which had previously received insulin produced less sugar than did the livers of untreated frogs Similar, though less well-controlled, results were obtained by Cori (29), Molitor and Pollak (30), and Sahyun (31) by different methods On the other hand, Lundsgaard *et al* (32, 33) were unable to show that insulin had any action on glycogen breakdown or deposition in the perfused livers of cats and dogs

More recently, however, Soskin and his co-workers (34, 35) were able to demonstrate an inhibitory effect of added glucose on the rate of appearance of free sugar in minced dog liver *in vitro* This offered the opportunity for the testing of the action of insulin on hepatic glycogenolysis under simplified conditions A lobe of the liver was removed from normal dogs anesthetized with nembutal Insulin was then administered to the animals, and 30–45 minutes later the remainder of the liver was removed In the liver samples removed after insulin administration, there was a significantly lower rate of appearance of free sugar than in the samples removed before insulin was given When glucose was added *in vitro* to both sets of liver samples, the rate of glycogenolysis was inhibited to a greater extent and by smaller amounts of added glucose in the "insulinized" samples than in the control samples (Table 26) It was apparent that insulin inhibited glycogenolysis in the liver and reinforced the inhibitory effect of added dextrose

The antiketogenic and nitrogen sparing effects of carbohydrate are ordinarily considered as requiring the presence of insulin, since they are difficult to elicit in its absence But the hormone is not essential, as has been shown by Soskin (36) In fact, this work demonstrated that every criterion of carbohydrate utilization which is exhibited by the normal animal can also be obtained without insulin in the completely depancreatized animal under the appropriate experimental conditions (see p 107) More recently, Mirsky and his co workers (37, 38) have shown that the antiketogenic and nitrogen sparing effects of carbohydrate can be obtained in acutely diabetic animals without insulin if the blood sugar is raised to a sufficiently high level Hence, the mode of action of insulin with respect to the foregoing he-

PHOSPHORYLATION, THE COMMON FACTOR IN INSULIN ACTION

It is a reasonable a priori assumption that the various physiological effects of insulin do not represent different and unrelated functions of the hormone It is

more likely that they are indirect consequences of a single catalytic influence on some basic enzyme system. From the functional standpoint the fundamental action of insulin may be considered as being the increased rate of entry of glucose (dextrose) from the blood and extracellular fluids into the tissue cells of the body This may not apply to organs like the brain and kidney; but it does apply at least to the skeletal muscles and liver, which compose the overwhelming bulk of the metabolically active tissues of the body In biochemical terms, the increased transit of sugar into the tissues may be described as the facilitation, by insulin, of a

TABLE 26

INHIBITION OF GLYCOGENOLYSIS IN LIVER BREI BY DEXTROSE AND BY DEXTROSE PLUS INSULIN (TAUBENHAUS *et al* [35])

Expt No	Time (Min)	Total Carbohydrate*	Amount of Dextrose Added*	Appearance of Free Sugar*		Percentage of Inhibition	
				Without Insulin	With Insulin	With Dextrose Alone	With Dextrose +Insulin
I	0	2,778	0	141			
	60		0	299			
	60		94	319	165	0	45
	60	2,897	186	267	145	11	52
II	0	3,313	0	254			
	60		0	1,997			
	60		100	2,027	1,180	0	42
	60		208	1,565	1,073	22	46
	60		418	1,229	805	39	60
	60	3,410	836	1,260	662	37	67
III	0	4,184	0	105	217		
	60		0	1,317	1,269		
	60		100	1,196	1,066	9	19
	60		200	1,080	892	18	32
	60		450	1,019	690	23	48
	60	4,000	900	446	222	66	83

* Values are in milligrams per 100 gm of liver calculated as for glucose

basic phosphorylation which introduces carbohydrate into the metabolic processes of the cell Regarded from the physical aspect, it may be said that, by increasing the rate of phosphorylation of glucose within the cell, insulin causes a steeper gradient of free sugar across the cell membrane and thus increases its rate of diffusion into the cell

As outlined in chapter iii, the present state of knowledge of the intermediate steps in carbohydrate metabolism indicates that the intermediate substance, the formation of which is facilitated by insulin, is one of the phosphorylated hexoses It will be recalled that the phosphorylation of sugar in the cell is accomplished by a substance possessing high-energy phosphate bonds, namely, adenosine triphos-

phate (ATP) The original energy necessary for the production of ATP from adenylic acid must eventually come from such oxidative reactions as may be coupled with the esterification of inorganic phosphate It must therefore be assumed that insulin acts at an as yet unknown locus in this cycle of events (39 40) This is consistent with the demonstrated effect of insulin in esterifying inorganic phosphate in the blood (p 172) It is also supported by the recent work of Sacks (41) with radioactive phosphorus in which he showed that insulin increased the rate of turnover of the phosphate in ATP

This hypothesis is compatible with the observed relationship between insulin action and sugar concentration It is to be expected that the rate of the basic phosphorylation like that of any other enzymatic reaction would be influenced by the concentration of the substrate Like any other catalyst insulin could be regarded as increasing the rate of the reaction for any given concentration of the substrate If the substrate concentration were high enough no additional effect of the catalyst could be demonstrated At low concentrations the action of the catalyst could be described as making possible rates of reaction which in the absence of the catalyst would require very high concentrations of substrate

From the foregoing point of view the various physiological effects of insulin which have been described as separate phenomena emerge as merely different parts of the same chain of events The fall in the blood sugar level is a direct reflection of the influence of insulin on the basic phosphorylation in so far as it causes a greater rate of removal of sugar from the blood The association of potassium with the hexose phosphates in muscle also accounts for the withdrawal of blood potassium The accelerated metabolic processes made possible by the increased rate of the first step in the series results in a greater disposal of the substrate both for synthetic and catabolic purposes (glycogen deposition and R Q change) The increased availability of the substrate to the enzymatic machinery of the cell allows carbohydrate to become predominant over protein and fat in the competition for the oxidative systems Hence the catabolism of protein and fat is inhibited (antiketogenesis and nitrogen sparing action) The latter effects are naturally prominent in the liver which is primarily concerned with the interconversion of foodstuffs while the former effects are more characteristic of the skeletal muscles and other effector organs which derive their energy chiefly from carbohydrate and ketoacids

INSULIN AND THE ENZYMATIC MACHINERY OF CARBOHYDRATE METABOLISM

The dominant role of ATP in tissue phosphorylations was described in chapter IV This high-energy phosphate compound is formed from adenylic acid and inorganic phosphate and the potential energy which it represents and which must be forthcoming for its continuous formation is presumably derived from oxidative steps in the dissimilation of carbohydrate (Fig 46) Using radioactive phosphorus

more likely that they are indirect consequences of a single catalytic influence on some basic enzyme system From the functional standpoint the fundamental action of insulin may be considered as being the increased rate of entry of glucose (dextrose) from the blood and extracellular fluids into the tissue cells of the body This may not apply to organs like the brain and kidney, but it does apply at least to the skeletal muscles and liver, which compose the overwhelming bulk of the metabolically active tissues of the body In biochemical terms, the increased transit of sugar into the tissues may be described as the facilitation, by insulin of a

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				Without Insulin	With Insulin	With Dextrose Alone	With Dextrose +Insulin
I	0	2 778	0	141			
	60		0	299			
	60		94	319	165	0	45
	60	2 897	186	267	145	11	52
II	0	3 313	0	254			
	60		0	1 997			
	60		100	2 027	1 180	0	42
	60		208	1 565	1 073	22	46
	60		418	1 229	805	39	60
	60	3 410	836	1 260	662	37	67
III	0	4 184	0	105	217		
	60		0	1 317	1 269		
	60		100	1 196	1 066	9	19
	60		200	1 080	892	18	32
	60		450	1 019	690	23	48
	60	4 000	900	446	222	66	83

* Values are in milligrams per 100 gm of liver calculated as for glucose

basic phosphorylation which introduces carbohydrate into the metabolic processes of the cell Regarded from the physical aspect, it may be said that by increasing *the rate of phosphorylation of glucose* within the cell, insulin causes a steeper gradient of free sugar across the cell membrane and thus increases its rate of diffusion into the cell

As outlined in chapter III the present state of knowledge of the intermediate steps in carbohydrate metabolism indicates that the intermediate substance, the formation of which is facilitated by insulin, is one of the phosphorylated hexoses It will be recalled that the phosphorylation of sugar in the cell is accomplished by a substance possessing high-energy phosphate bonds, namely, adenosine triphos

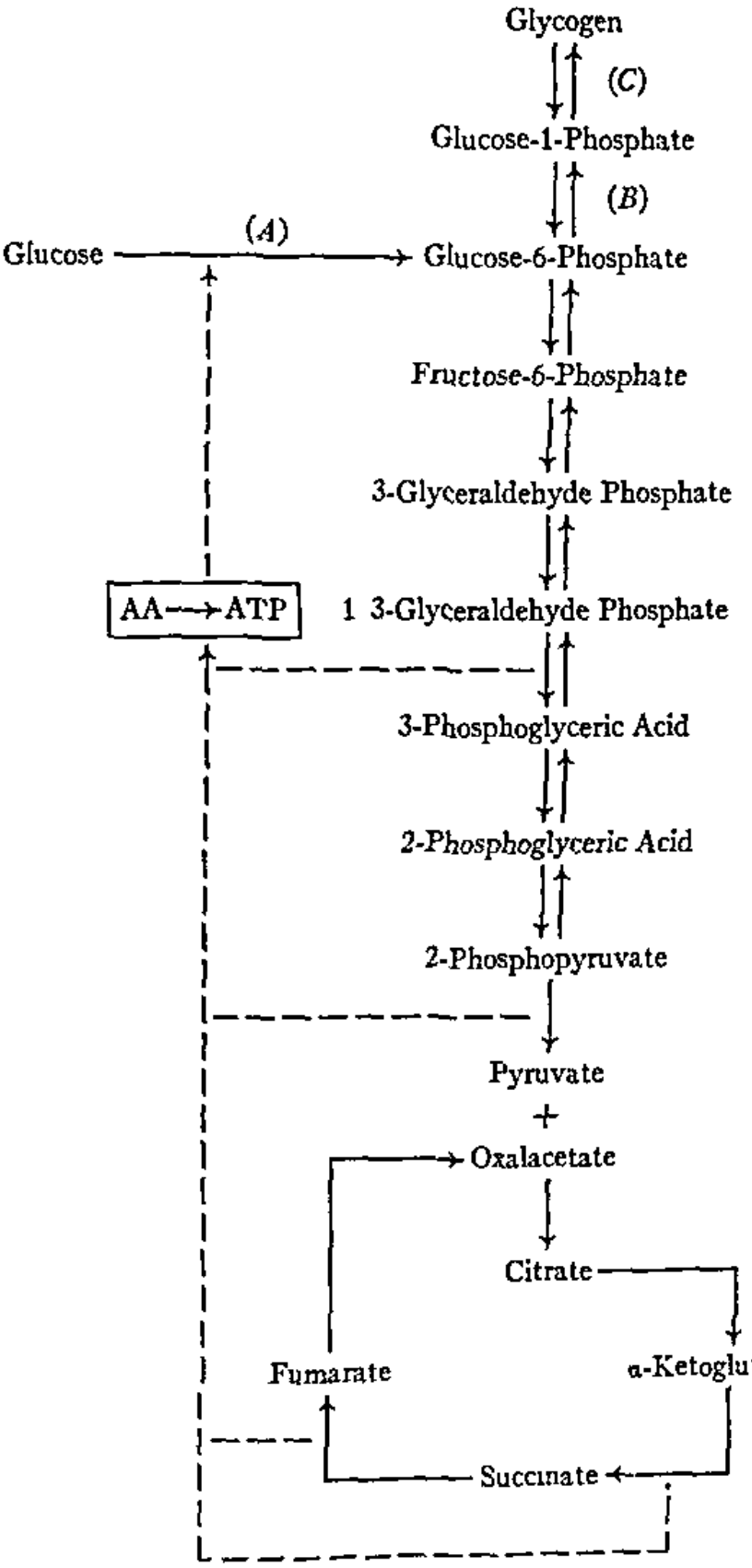
phate (ATP) The original energy necessary for the production of ATP from adenylic acid must eventually come from such oxidative reactions as may be coupled with the esterification of inorganic phosphate It must therefore be assumed that insulin acts at an as yet unknown locus in this cycle of events (39 40) This is consistent with the demonstrated effect of insulin in esterifying inorganic phosphate in the blood (p 172) It is also supported by the recent work of Sacks (41) with radioactive phosphorus in which he showed that insulin increased the rate of turnover of the phosphate in ATP

This hypothesis is compatible with the observed relationship between insulin action and sugar concentration It is to be expected that the rate of the basic phosphorylation like that of any other enzymatic reaction would be influenced by the concentration of the substrate Like any other catalyst, insulin could be regarded as increasing the rate of the reaction for any given concentration of the substrate If the substrate concentration were high enough no additional effect of the catalyst could be demonstrated At low concentrations the action of the catalyst could be described as making possible rates of reaction which in the absence of the catalyst would require very high concentrations of substrate

From the foregoing point of view the various physiological effects of insulin which have been described as separate phenomena emerge as merely different parts of the same chain of events The fall in the blood sugar level is a direct reflection of the influence of insulin on the basic phosphorylation in so far as it causes a greater rate of removal of sugar from the blood The association of potassium with the hexose phosphates in muscle also accounts for the withdrawal of blood potassium The accelerated metabolic processes made possible by the increased rate of the first step in the series results in a greater disposal of the substrate *both for synthetic and catabolic purposes* (*glycogen deposition* and *R Q* change) The increased availability of the substrate to the enzymatic machinery of the cell allows carbohydrate to become predominant over protein and fat in the competition for the oxidative systems Hence the catabolism of protein and fat is inhibited (antiketogenesis and nitrogen sparing action) The latter effects are naturally prominent in the liver which is primarily concerned with the interconversion of foodstuffs while the former effects are more characteristic of the skeletal muscles and other effector organs which derive their energy chiefly from carbohydrate and ketoacids

INSULIN AND THE ENZYMATIC MACHINERY OF CARBOHYDRATE METABOLISM

The dominant role of ATP in tissue phosphorylations was described in chapter IV This high-energy phosphate compound is formed from adenylic acid and inorganic phosphate and the potential energy which it represents and which must be forthcoming for its continuous formation is presumably derived from oxidative steps in the dissimilation of carbohydrate (Fig 46) Using radioactive phosphorus



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as a tracer, Sacks (41) has demonstrated a more rapid turnover of ATP in the skeletal muscle of intact animals when glucose and insulin were administered than when glucose alone was given Since the rate of phosphorylation of glucose depends upon the rate of turnover of ATP, it is obvious that insulin might act on any of the oxidative reactions that supply the energy for the rephosphorylation of adenylic acid But the fact that insulin does not increase oxygen consumption, either *in vivo* (42) or *in vitro* (Table 27), makes this simple explanation untenable This anomalous situation might be resolved by supposing that, without actually increasing the rate of oxidative reactions, insulin increased their efficiency as regards phosphorylation so that more moles of inorganic phosphate were esterified per mole of oxygen consumed (40) This is not unreasonable, in view of the fact that different investigators have reported various ratios of phosphate esterifica

TABLE 27

LACK OF EFFECT OF INSULIN ON THE OXYGEN CONSUMPTION OF MAMMALIAN MUSCLE *in vitro*

Condition of Animal	Type of Tissue	Glucose in Medium (Mg per Cent)	Insulin	Q_{O_2}	Reference
Normal	Abdominal muscle	400	0	3 0	Levine *et al* (39)
		0	0	2 9	
		0	+	3 0	
		400	+	3 1	
Normal	Diaphragm	0	0	4 7	Gemmill (20)
		200	0	4 7	
		500	0	5 3	
		500	+	4 7	

tion to oxygen consumption according to the experimental conditions which they employed

Considering the fact that insulin usually raises the R Q without affecting the oxygen consumption, one might suppose that insulin acted on some as yet unknown non oxidative decarboxylation But this could hardly be a direct or essential part of insulin action, in view of certain of Gemmill s results (Table 28) It may be seen that he was able to demonstrate a very significant action of insulin as regards glycogen deposition with no appreciable influence on the R Q

Of course insulin might act higher up in the scheme of dissimulation and be concerned either with the enzyme acting directly on glucose (Fig 46 step *A*) or with the systems between glucose 6-phosphate and glycogen (Fig 46 steps *B* and *C*) It has been possible to test the latter systems with purified enzymes *in vitro*, and the results have been negative as regards any effect of insulin (3, 6) It has likewise been shown that in the absence of added glucose insulin has no effect upon the

rate of glycogen breakdown in mammalian muscle *in vitro* (Fig 47) Unfortunately, it has thus far been impossible to obtain an extract of skeletal muscle which will phosphorylate glucose *in vitro* An enzyme obtained from yeast and known as "hexokinase" will do so, but it is not influenced by insulin However, hexokinase need not be similar to the enzyme system responsible for glucose phosphorylation in mammalian muscle, for, while hexokinase will phosphorylate fructose even

TABLE 28

INFLUENCE OF INSULIN IN INCREASING THE DEPOSITION OF GLYCOGEN IN RAT DIAPHRAGM *in vitro* WITHOUT AFFECTING THE R Q SIGNIFICANTLY

Glucose in Medium (Mg per Cent)	Insulin	Q_{O_2}	Total Carbohydrate Change in Tissue (Mg/100 Mg)	R Q	Reference
0	0	4 8	−0 09	0 73	Gemmill (19 20)
200	0	4 9	+0 37	0 86	
200	+	4 6	+0 82	0 91	
500	0	5 2	+0 56	0 86	
500	+	4 7	+1 18	0 88	

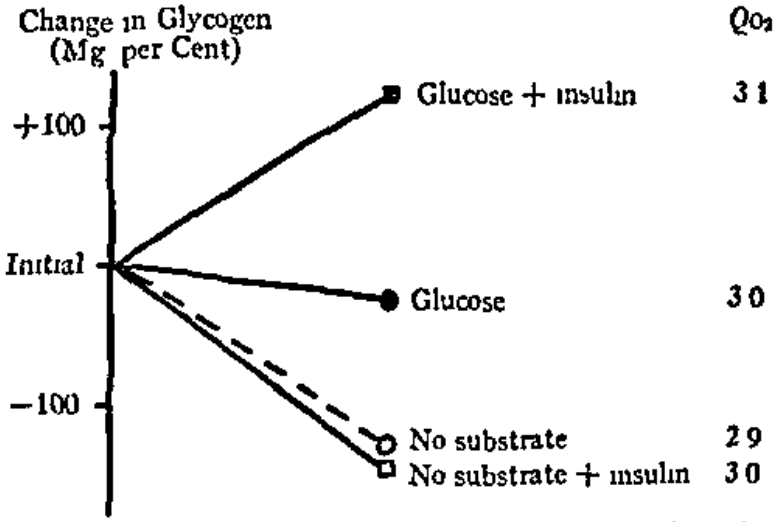

FIG 47 —Lack of influence of insulin on glycogen content of rat abdominal muscle *in vitro* in the absence of glucose (Levine *et al* [39])

more readily than it does glucose, mammalian skeletal muscle *in vitro* will deposit glycogen only from glucose and not from fructose, mannose, or galactose (Fig 48) Until the glucose-phosphorylating enzyme of muscle is isolated, it will be impossible to decide whether or not insulin may act at this point

Another difficulty is our present uncertainty as to the correctness of some of the details in our conception of carbohydrate dissimilation as outlined in Figure 46 For example, in the same experiments in which Sacks (41) showed that insulin in

creased the rate of turnover of ATP, he was unable to find any corresponding in crease in the rate of turnover of glucose 6-phosphate This may mean that, in skeletal muscle, glucose is phosphorylated to glucose 1 phosphate rather than to glucose-6 phosphate If this were so, muscle would differ from brain, liver, and kidney, the extracts of which have been shown to phosphorylate glucose to glu cose 6-phosphate

Data of more positive significance indicate that the point of action of insulin is probably above pyruvate In the presence of sodium fluoride, which inhibits glycolysis at the phosphoglyceric acid stage, the addition of insulin to muscle *in vitro* still leads to a greater esterification of inorganic phosphate (Fig 49) A similar significance may be attached to the recent work of Himwich *et al* (27) on depancreatized dogs They found a greater rise of pyruvic acid in the blood after

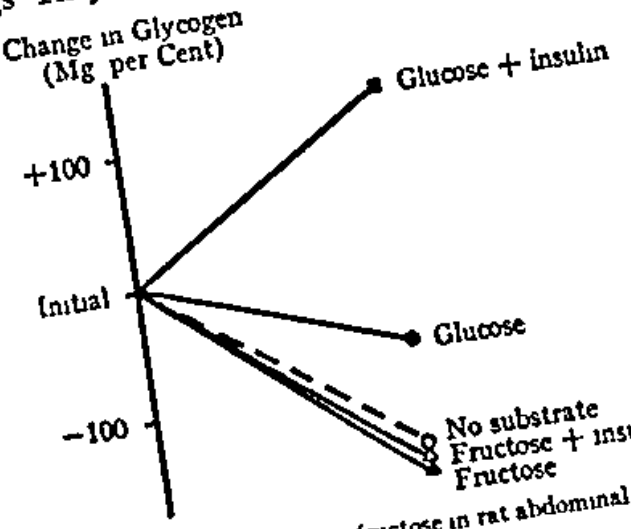

Fig 48 —Lack of formation of glycogen from fructose in rat abdominal muscle *in vitro* with or without insulin (Levine *et al* [39])

the administration of glucose plus insulin than resulted from the giving of the same amount of glucose alone

The work of Bach and Holmes (43) on liver slices *in vitro* in which they demonstrated that insulin inhibited deamination suggests a locus of action of insulin entirely outside of carbohydrate metabolism Taken at its face value, this work could mean either that insulin has more than one fundamental action or that it affects protein metabolism directly and carbohydrate metabolism only indirectly However, it seems more likely that the reverse of the latter is the case Insulin may produce this effect not by any direct action on the amino acid oxidase but by increasing the rate of entry of carbohydrate into the metabolic cycle

As regards a possible direct influence of insulin on fat metabolism, which might be predicated on the basis of its notable antiketogenic action in the intact animal, there is no pertinent *in vitro* work available The enzyme systems concerned with fat metabolism are almost completely unknown

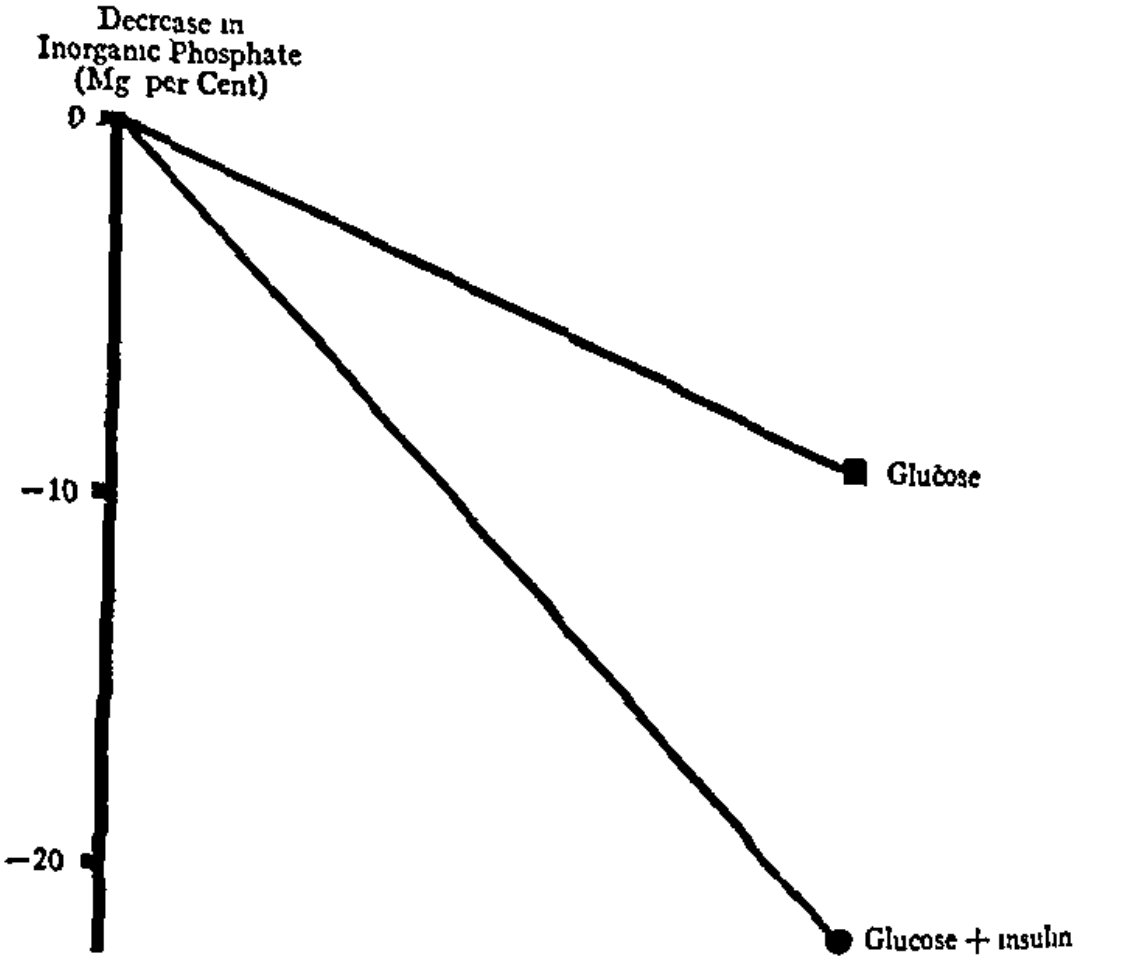

FIG 49 —Influence of insulin on the decrease in inorganic phosphate (esterification) in rat abdominal muscle *in vitro* (Levine *et al* [39])

TABLE 29

LACK OF SIGNIFICANT DIFFERENCE BETWEEN NORMAL AND DIABETIC MUSCLE *in vitro*

For the respiratory experiments intact abdominal muscle of young rats (60–80 gm) was used The phosphate partitions were determined on the gastrocnemii of the same animals P_a = inorganic phosphate, P_y = two thirds of adenosine polyphosphate, P Creat = creatine phosphate, P Total = total acid soluble phosphate

CONDITION	NO OF ANIMALS	BLOOD SUGAR (MG PER CENT)	Q_{O_2}	R Q	LACTIC ACID PRODUCTION (MG PER 100 GM PER HR)		PHOSPHATE PARTITION (MG PER CENT)			
					In O_2	In N_2	P_a	P_y	P Creat	P Total
Normal	10	123	3 8	0 81	78	355	17	32	55	139
Diabetic (alloxan)	15	393	3 2	0 78	83	278	22	34	57	143

It is evident that while we are perhaps closer to the solution of insulin action than we are to the action of any other hormone, the problem is far from solved It may be that the failure to arrive at the ultimate solution depends upon the fact that very little *in vitro* work has been done with the tissues of diabetic animals We have seen that in the normal intact animal an optimal amount of insulin, as regards glucose utilization, is present, so that the administration of additional insulin is without influence in this respect It does increase glycogen deposition in the muscles and this effect can also be demonstrated in isolated normal muscle *in vitro* Further *in vitro* investigations using diabetic instead of normal tissues might be fruitful as regards other influences of insulin Certainly, not much progress was possible in the search for the points of action of the various components of the vitamin B complex on the enzymatic machinery of metabolism until the tissues of animals deprived of specific vitamins became available However, the authors must confess that their own *in vitro* studies with diabetic mammalian muscle have not been very enlightening thus far Table 29 indicates a number of respects in which the diabetic muscle does not appear to differ from normal muscle

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CHAPTER XVII

THE ADRENAL CORTEX

THE essential nature of the adrenal glands to the well being of man was first indicated in Addison's original description (1) of the disease which has since been called by his name The influence of the gland on carbohydrate metabolism was for a long time ascribed to the secretion of the adrenal medulla In 1909 Porges (2, 3) reported the occurrence of hypoglycemia in Addison's disease, which was by that time recognized as primarily affecting the adrenal cortex He also demonstrated the occurrence of low carbohydrate levels in bilaterally adrenalectomized dogs Despite subsequent substantiation of these findings, little advance in knowledge as to the carbohydrate functions of the adrenal cortex was made until the early work of Britton and his co-workers (4, 5, 6)

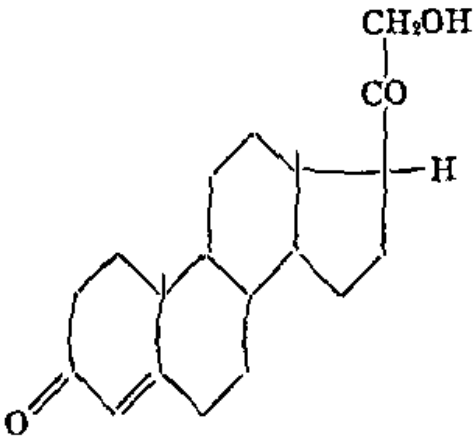
Stewart and Rogoff (7) had previously made adrenal cortical extracts capable of maintaining the life of adrenalectomized animals Swingle and Pfiffner (8, 9) devised a new method for extraction but were particularly struck by the influence of their extract on salt and water metabolism Britton and his co workers (5, 10, 11), on the other hand, emphasized the importance of hypoglycemia and low glycogen levels as factors leading to the death of their adrenalectomized animals While they also observed certain effects on the sodium and potassium levels of the blood, they insisted that the prepotent influence of their extracts was exerted on carbohydrate metabolism

The controversial nature of the subject gradually abated as it became apparent that both the mineral and carbohydrate effects were salient features of adrenalectomy, that they could be obtained with adrenal cortical extracts, and that they were not completely independent of each other The use of depancreatized and of hypophysectomized animals facilitated the establishment of the carbohydrate functions of the adrenal cortex, and, finally, the potent steroids, separated from the extracts by Reichstein (12) and by Kendall (13), have made possible the accumulation of data on each aspect of adrenal function, uncomplicated by the other

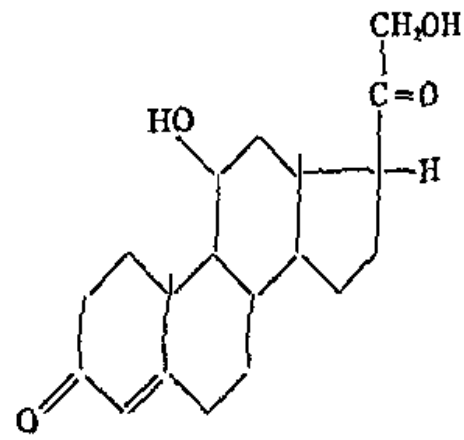
THE STEROIDS OF THE ADRENAL CORTEX

The attempts at isolation of the adrenal cortical hormone have made it sufficiently evident that, whatever its natural structure, extracts of the gland may not be regarded as containing a single active substance Hartman *et al* (14) have reported that they find two factors in adrenal cortical extracts which potentiate each other

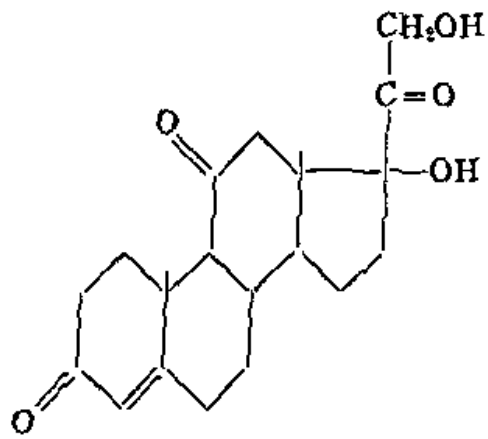
but which have largely separate actions One maintains the sodium levels of the tissues but is relatively ineffective in maintaining appetite and normal behavior and in preserving life in adrenalectomized cats The other factor ("cortin") is very potent in preserving life, appetite, weight, and normal behavior even while the serum sodium remains low In the light of other work, however, the views of Hartman *et al* would seem to represent an oversimplification of the problem and to



DESOXYCORTICOSTERONE



CORTICOSTERONE



17 HYDROXYDEHYDROCORTICOSTERONE

FIG 50 —Representative steroids of the adrenal cortex

minimize the importance of the sodium and potassium balance for the well being of the living organism

The isolation and identification of a number of steroids (13 15, 16) from the adrenal cortex and the study of their physiological properties and those of the amorphous fractions have revealed that the various compounds or fractions have certain activities in common Figure 50 shows the formulas of some representative cortical steroids However, a particular compound or fraction may exhibit one activity to the highest degree and be relatively impotent in other respects In the ab

sence of more precise knowledge of that vital function, the failure of which is the most urgent cause of death in untreated adrenalectomized animals, it is convenient to compare the various cortical steroids and fractions in regard to the following effects on such animals (*a*) the maintenance of life, (*b*) the restoration of normal carbohydrate levels in all tissues, and (*c*) the restoration of normal sodium and potassium balance and excretion To these effects may be added the restoration of the ability of the muscles to continue to perform work in response to prolonged stimulation, according to the test developed by Ingle (17) But since the activities of substances in this respect run parallel with their carbohydrate effects, these two actions may be considered together

Kendall s amorphous fraction (cortin) and his desoxy B compound seem to be the most potent for maintaining life (18, 19) The carbohydrate levels are best restored by corticosterone and its derivatives which have an oxygen or hydroxyl group on C_{11} (19, 20) In this respect, cortin has some effect, but desoxycorticosterone has very little (21) The relative potencies of the substances acting on carbohydrate levels maintain a similar relationship when these materials are tested on muscular work performance (19, 22) Some of the earlier work with synthetic desoxycorticosterone acetate, while showing its powerful influence on the sodium and potassium balance, had revealed no action on carbohydrate metabolism (23, 24) This is apparently a matter of dosage, for Harrison and Harrison (25) have reported that 1 25 mg daily of the substance would maintain life and a normal mineral balance in adrenalectomized rats but that it required 2 5 mg daily to maintain a normal blood sugar level Similar evidence is available in the work of Britton and Corey (26), Ingle (27), Wells (28, 29), and Long, Katzin, and Fry (21), although these authors differ from Harrison and Harrison and from each other as to the comparative potency of desoxycorticosterone on carbohydrate metabolism

Table 30, which modifies and amplifies one of Ingle's (19), summarizes the relative quantitative effects of salt and of steroids, which have been shown to substitute for the functional activity of the adrenal cortex in one respect or another

DEFICIENCES RELIEVED BY SALT TREATMENT

In spite of the qualitative difference in the prepotent activity of the various substances which may be separated from adrenal cortical extracts, it is impossible to discuss the materials concerned with the metabolism of the foodstuffs without also considering those which primarily affect the mineral balance This is because the absence of the latter in adrenalectomized animals disturbs the normal environment of all cells and thus produces certain secondary disturbances in metabolism The secondary effects are most readily distinguished from the primary metabolic effects of adrenalectomy by a consideration of those disturbances which are alleviated by combating the mineral imbalance with a high sodium and low potassium

intake The symptoms of adrenal cortical insufficiency which are relieved by salt treatment are as follows

1 *Decrease in the sodium content and increase in the potassium of the blood serum* — This is accompanied by an increased excretion of sodium in the urine and a decreased excretion of potassium (30, 31) The changes in excretion are known to be due to a specific effect upon the kidney tubules (32) The changes in the blood levels are due partly to disturbed kidney function and partly to a similar derangement of electrolyte balance in the other tissues of the body (33, 34)

2 *Dehydration and hemoconcentration* —These are secondary to the loss of H_2O involved in the excessive excretion of NaCl They are partly responsible for the

TABLE 30

DEGREE OF RESTORATION TO NORMAL OF THE EFFECTS OF ADRENALECTOMY BY VARIOUS MODES OF SUBSTITUTION THERAPY

(++++ = Complete Restitution)

CONDITION	DEGREE OF RESTORATION BY—		
	NaCl	Desoxycorticosterone	C. Steroids
Low blood NaCl	++++	++++	++
High blood potassium	++++	++++	++
Survival on food	++++	++++	
Low basal metabolic rate	++++	++++	
High blood urea	+++	+++	++
Low carbohydrate absorption	++++	++++	
Survival on fasting	+++	+++	++++
Lowered storage of fed glucose	+++	+++	++++
Low resistance to stress	+	+	+++
Low nitrogen excretion on fasting	++		++++
Work performance	+	+	++++
Insulin sensitivity	+	+	++++
Low carbohydrate levels on fasting	+	++	++++
Reduction of diabetic hyperglycemia and glycosuria	+	++	++++

rise in blood urea, although the disturbance in kidney function also contributes to this effect (34 35)

3 *Acidosis* —This is due to the retention of acid metabolites and anions, which are ordinarily neutralized and excreted by the kidneys The failure in excretion is due in part to the circulatory failure and in part to the specific kidney disturbance A feature of the latter is an inability to produce NH_3 for the regulation of the acid base balance

4 *Impairment of carbohydrate absorption by the gastro intestinal tract and of the glycogen deposition from ingested carbohydrates* —These effects may be related to the movement of potassium out of all tissue cells Fenn showed that the passage of sugar into the cell was accompanied by a movement of potassium in the same direction (36)

5 *Decreased metabolic rate* —This has been demonstrated for the isolated tis sues of adrenalectomized animals *in vitro* (37, 38) In the living animal it may also depend upon the reduced blood chloride level, which interferes with the dissocia tion of oxygen from oxyhemoglobin, decreasing the supply of oxygen to the tis sues (39, 40)

6 *Anorexia and the consequent lack of gain in weight and cessation of growth* — No explanation for the loss of appetite is available

7 *Rapid deterioration and death of the animal* —This is probably a result of the cumulative effects of dehydration and hemoconcentration leading to a shocklike condition, plus the toxic action of high potassium levels and the hypoglycemic ef fects of fasting owing to the anorexia

The beneficial effects of salt on the above symptoms are striking and very read ily demonstrated The diminished rate of glucose absorption is completely re stored to normal by the administration of NaCl in the drinking water (41, 42) The same holds true for fat absorption (43) Similarly treated adrenalectomized rats can deposit glycogen from glucose nearly as well as normal rats (42 44) and may gain weight in normal fashion (45) But while salt treatment enables adrenalectomized animals to survive indefinitely under favorable conditions it does not restore them completely to normal They are still sensitive to stresses and strains of all kinds (19 44) Nor is this sensitivity completely abolished by treatment with the ster oids that are active as regards carbohydrate metabolism (19 34) It is upon this evidence that the possibility of the existence of a separate life maintaining" prin ciple is based (19)

The observations of the normal absorption of carbohydrate and fat in salt treated adrenalectomized animals (44) are directly opposed to the theories of Ver zar This author, starting with his observation that the intestinal absorption of the foodstuffs was diminished after adrenalectomy had related this defect to a dis turbance of the phosphorylating mechanisms and had assembled rather impressive evidence that the adrenal cortex was primarily concerned with phosphate transfer Recent attempts to confirm his findings and conclusions have been almost uniform ly unsuccessful (46 47)

DEFICIENCIES RELIEVED BY THE C_{21} STEROIDS

What, then are the primary functions of the adrenal cortex in respect to the metabolism of the foodstuffs? The answer appears in those metabolic disturbances in the adrenalectomized animal which persist despite the maintenance of a normal sodium and potassium balance These include

1 *Hypoglycemic effect of fasting* —Salt treated animals which appear perfectly normal and healthy when maintained on an ample diet rapidly deteriorate when food is withdrawn dying in hypoglycemia (21, 24, 34) The administration of sugar (*in physiological saline*) rapidly restores them

2 *Reduced levels of tissue glycogen, particularly that of liver glycogen, during fasting* —This is due to an inability to manufacture glycogen from the body stores of non-carbohydrate precursors and accounts also for the hypoglycemic effect of fasting (19 21, 34, 48)

3 *Diminished urinary nitrogen excretion during fasting* —In view of the fact that the protein fed adrenalectomized animal excretes normal amounts of nitrogen (21, 49), it seems likely that the difficulty in the fasted adrenalectomized animal is that of mobilization of protein from the tissues and its breakdown to the amino acid stage

4 *Disturbance in fat mobilization* —Anterior pituitary extracts (50), phlorhizin administration (51), or phosphorus poisoning (51) result in the accumulation of fat in the livers of normal animals but fail to do so in the absence of the adrenals

5 *Alleviation of experimental diabetes* —The diminution of hyperglycemia, glycosuria and ketosis in depancreatized and phlorhizinized animals which lack the adrenal cortex is readily explained by the disturbances in the mobilization of protein and fat and the consequent dearth of raw materials for gluconeogenesis (19 21, 48 52, 53)

6 *Insulin sensitivity* —This is not due to the lack of available liver glycogen to combat hypoglycemia, for the salt treated adrenalectomized animal with a fairly normal hepatic glycogen level still exhibits the sensitivity (48, 54, 55)

7 *Muscular weakness* —This is alleviated by the administration of carbohydrate (19, 56)

Treatment of fasting adrenalectomized animals with corticosterone or cortin (19 21, 34) restores the normal blood sugar level and, in large doses, may cause hyperglycemia (see Table 31) Such treatment also increases the liver glycogen in normal, as well as in adrenalectomized, animals (19, 21) The muscle glycogen is not so readily affected either by adrenalectomy or by the administration of cortical extracts Recent work has also confirmed the previous reports that the lack of adrenal cortical hormone diminishes the hyperglycemia and glycosuria of diabetes (21, 48, 52) and that the administration of active cortical hormones restores the severity of the diabetic syndrome (28) Sprague *et al* (57) have reported a case of a typical diabetes mellitus in a woman which disappeared completely upon the removal of an adrenal cortical tumor

Wells (28) has reported that the injection of phlorhizin into salt treated adrenalectomized rats causes them to excrete much smaller amounts of glucose than similarly injected normal rats Corticosterone and 17 hydroxy 11-dehydrocorticosterone (Compound E) increase the glucose excretion of the phlorhizinized adrenalectomized animals to that of phlorhizin treated normal rats The amorphous fraction (cortin) and desoxycorticosterone have relatively lesser effects (see Table 32)

It may therefore be concluded that the primary metabolic functions of the

adrenal cortex are concerned with hepatic gluconeogenesis from non-carbohydrate precursors The observation of Corey and Britton (58) that cortical extracts retard the fall of glycogen in perfused livers also suggests an antiglycogenolytic activity of the adrenal cortex This may explain the more marked effects of cortical extracts on liver glycogen, as compared to muscle glycogen It also helps to distinguish the action of these extracts from those of the anterior hypophysis (59) (see chap xix, p 225)

TABLE 31

EFFECTS OF ADRENALECTOMY AND OF CORTICAL STEROIDS ON THE CARBOHYDRATE LEVELS OF RATS AND MICE (LONG *et al* [21])

Species	Condition	Hormonal Therapy	Blood Sugar (Mg per Cent)	Liver Glycogen (per Cent)	Muscle Glycogen (Mg per Cent)
Rats	Normal—fed	0	124	1 78	590
	Normal—48 hr fast	0	80	0 23	507
	Normal—48 hr fast	Cortical extract		1 64	536
	Adrenalectomy—fed	0	97	2 31	533
	Adrenalectomy—48 hr fast	0	30	0 07	358
	Adrenalectomy—48 hr fast	Cortical extract		1 78	411
Mice	Normal—fed	0		2 84	435
	Normal—fed	Cortical extract		9 90	1,014
	Normal—24 hr fast	0		0 35	228
	Normal—24 hr fast	Cortical extract		2 99	223
	Normal—24 hr fast	Corticosterone		1 89	
	Normal—24 hr fast	Dehydrocorticosterone		2 26	
	Adrenalectomy—fed	0		2 18	479
	Adrenalectomy—24 hr fast	0		0 04	158
	Adrenalectomy—24 hr fast	Cortical extract		2 37	182

MODE OF ACTION OF THE C_{21} STEROIDS ON CARBOHYDRATE METABOLISM

From their observations on the effect of cortical extract on the R Q of glucose-fed adrenalectomized animals, Long Russell, and others (48, 60, 61) have supposed that the adrenal cortical hormone may depress carbohydrate 'oxidation" This conclusion is subject to the usual objections which apply to such use of the R.Q (62) Moreover, Selye and Dosne (63) have shown that, while cortical extract will inhibit the fall in blood sugar of partially hepatectomized rats, it fails to have any effect in completely liverless animals (confirmed by Reinecke [64]) Concordant evidence in patients suffering from Addison's disease was reported by McBryde and De la Balze (75), who found a very significant increase in the arteriovenous blood sugar difference after treatment with cortical extract rich in the C_{21} steroids,

2 *Reduced levels of tissue glycogen, particularly that of liver glycogen, during fasting* —This is due to an inability to manufacture glycogen from the body stores of non carbohydrate precursors and accounts also for the hypoglycemic effect of fasting (19, 21, 34, 48)

3 *Diminished urinary nitrogen excretion during fasting* —In view of the fact that the protein fed adrenalectomized animal excretes normal amounts of nitrogen (21, 49), it seems likely that the difficulty in the fasted adrenalectomized animal is that of mobilization of protein from the tissues and its breakdown to the amino acid stage

4 *Disturbance in fat mobilization* —Anterior pituitary extracts (50), phlorhizin administration (51), or phosphorus poisoning (51) result in the accumulation of fat in the livers of normal animals but fail to do so in the absence of the adrenals

5 *Alleviation of experimental diabetes* —The diminution of hyperglycemia, glycosuria and ketosis in depancreatized and phlorhizinized animals which lack the adrenal cortex is readily explained by the disturbances in the mobilization of protein and fat and the consequent dearth of raw materials for gluconeogenesis (19 21, 48, 52, 53)

6 *Insulin sensitivity* —This is not due to the lack of available liver glycogen to combat hypoglycemia, for the salt treated adrenalectomized animal with a fairly normal hepatic glycogen level still exhibits the sensitivity (48, 54, 55)

7 *Muscular weakness* —This is alleviated by the administration of carbohydrate (19, 56)

Treatment of fasting adrenalectomized animals with corticosterone or cortin (19 21, 34) restores the normal blood sugar level and, in large doses, may cause hyperglycemia (see Table 31) Such treatment also increases the liver glycogen in normal, as well as in adrenalectomized, animals (19 21) The muscle glycogen is not so readily affected either by adrenalectomy or by the administration of cortical extracts Recent work has also confirmed the previous reports that the lack of adrenal cortical hormone diminishes the hyperglycemia and glycosuria of diabetes (21, 48, 52) and that the administration of active cortical hormones restores the severity of the diabetic syndrome (28) Sprague *et al* (57) have reported a case of a typical diabetes mellitus in a woman which disappeared completely upon the removal of an adrenal cortical tumor

Wells (98) has reported that the injection of phlorhizin into salt treated adrenalectomized rats causes them to excrete much smaller amounts of glucose than similarly injected normal rats Corticosterone and 17 hydroxy 11-dehydrocorticosterone (Compound E) increase the glucose excretion of the phlorhizinized adrenalectomized animals to that of phlorhizin treated normal rats The amorphous fraction (cortin) and desoxycorticosterone have relatively lesser effects (see Table 32)

It may therefore be concluded that the primary metabolic functions of the

adrenal cortex are concerned with hepatic gluconeogenesis from non carbohydrate precursors The observation of Corey and Britton (58) that cortical extracts retard the fall of glycogen in perfused livers also suggests an antiglycogenolytic activity of the adrenal cortex This may explain the more marked effects of cortical extracts on liver glycogen, as compared to muscle glycogen It also helps to distinguish the action of these extracts from those of the anterior hypophysis (59) (see chap xix, p 225)

TABLE 31

EFFECTS OF ADRENALECTOMY AND OF CORTICAL STEROIDS ON THE CARBOHYDRATE LEVELS OF RATS AND MICE (LONG *et al* [21])

Species	Condition	Hormonal Therapy	Blood Sugar (Mg per Cent)	*Liver* Glycogen (per Cent)	Muscle Glycogen (Mg per Cent)
Rats	Normal—fed	0	124	1 78	590
	Normal—48 hr fast	0	80	0 23	507
	Normal—48 hr fast	Cortical extract		1 64	536
	Adrenalectomy—fed	0	97	2 31	533
	Adrenalectomy—48 hr fast	0	30	0 07	358
	Adrenalectomy—48 hr fast	Cortical extract		1 78	411
Mice	Normal—fed	0		2 84	435
	Normal—fed	Cortical extract		9 20	1,014
	Normal—24 hr fast	0		0 35	228
	Normal—24 hr fast	Cortical extract		2 99	223
	Normal—24 hr fast	Corticosterone		1 89	
	Normal—24 hr fast	Dehydrocorticosterone		2 26	
	Adrenalectomy—fed	0		2 18	479
	Adrenalectomy—24 hr fast	0		0 04	158
	Adrenalectomy—24 hr fast	Cortical extract		2 37	182

MODE OF ACTION OF THE C_{21} STEROIDS ON CARBOHYDRATE METABOLISM

From their observations on the effect of cortical extract on the R Q of glucose fed adrenalectomized animals, Long, Russell, and others (48, 60, 61) have supposed that the adrenal cortical hormone may depress carbohydrate "oxidation" This conclusion is subject to the usual objections which apply to such use of the R Q (62) Moreover, Selye and Dosne (63) have shown that, while cortical extract will inhibit the fall in blood sugar of partially hepatectomized rats, it fails to have any effect in completely liverless animals (confirmed by Reinecke [64]) Concordant evidence in patients suffering from Addison's disease was reported by McBryde and De la Balze (75), who found a very significant increase in the arteriovenous blood sugar difference after treatment with cortical extract rich in the C_{21} steroids,

despite the fact that this treatment undoubtedly increases the rate of circulation It is apparent, therefore, that cortical extract does not inhibit the uptake of sugar by the peripheral tissue but probably stimulates gluconeogenesis in the liver It is suggested that its tendency to counteract insulin hypoglycemia (54 55) is exerted in a similar manner

TABLE 32

EFFECT OF PHLORHIZIN UPON THE EXCRETION OF DEXTROSE AND NITROGEN BY RATS UNDER VARYING CONDITIONS OF ENDOCRINE ABLATION AND SUBSTITUTION THERAPY*

ENDOCRINE STATE	SUBSTITUTION THERAPY	DEXTROSE (MG PER 100 GM PER DAY)	NITROGEN (MG PER 100 GM PER DAY)	D N	COMPARATIVE EXCRETION (PER CENT OF NORMAL)	
					Dextrose	Nitrogen
Normal		621 574	182 162	3 4 3 5	100	100
Adrenal demedullation		624	172	3 6	104	100
Adrenalectomy	NaCl	142	46	3 7	24	27
	Desoxycorticosterone	440	124	3 3	74	72
	Corticosterone	590	165	3 6	98	93
	Compound E	619 560	190 155	3 3 3 6	98	100
	Amorphous fraction	237	63	3 8	40	37
Thyroidectomy		477	139	3 4	80	81
Adrenalectomy and thyroidectomy		140	61	2 3	23	35
	Compound E	382	103	3 7	64	60
	Compound E+thyroxin	721	190	3 8	121	114
Hypophysectomy		148	57	2 6	25	33
	Desoxycorticosterone	323	100	3 2	54	58
	Corticosterone	449	158	2 8	75	93
	Compound E	412	170	2 4	69	99
	Compound E+thyrotrophic hormone	625	196	3 2	105	114

* These data are derived from the papers of Wells Kendall and associates (28 29 49 73 74)

The probability that the low carbohydrate levels in the fasting adrenalectomized animal are not due to an increased carbohydrate "oxidation" is enhanced by the demonstration of an impaired work performance of the muscles Ingle(19) has shown that the work performance is markedly diminished in adrenalectomized animals, even when they are maintained in apparently good condition by a diet high in sodium and low in potassium This effect is due wholly to the loss of the adrenal cortex for removal of the adrenal medulla has no influence (65) The po

tency of various cortical steroids in restoring the ability of the muscles to do work is parallel with their potency as regards carbohydrate metabolism (see Table 30) Ingle has also shown that the work performance is restored to normal by the ad ministration of glucose in the absence of cortical compounds These observations would present a curious anomaly if one were to accept the conclusions of Long and co workers as regards the increased ' oxidation' of carbohydrate in adrenalecto mized animals and its suppression by cortical hormones One would have to recon cile the facts that both the administration of cortical steroids which supposedly suppress glucose "oxidation ' and the administration of glucose itself lead to a res toration of normal work performance

The manner in which the adrenal cortex stimulates hepatic gluconeogenesis is by no means clear, but evidence is forthcoming that it influences the mobilization and catabolism of both protein and fat Nitrogen excretion is decreased following adrenalectomy, and the administration of cortical extracts restores the nitrogen output to normal The increased glycosuria observed after the treatment of adre nalectomized depancreatized animals with cortical fractions or steroids is accom panied by a corresponding increase in the urinary nitrogen Wells *et al* (28) have demonstrated similar effects with the cortical substances in phlorhizinized adrenal ectomized rats (Table 32) Another observation which is consistent with the cata bolic effect of the adrenal cortex on protein metabolism is that of Fraenkel Conrat *et al* (66), who showed that adrenal cortical extracts or the adrenotrophic fraction of the anterior pituitary cause an increase in the level of liver arginase, an enzyme which is concerned in the formation of urea from amino acids (67)

Concerning the mobilization of fat it had been shown that the phospholipids and fatty acids of the blood were decreased following adrenalectomy (68) and that various procedures which increased the fat content of the liver in normal animals usually failed to do so in the absence of the adrenals (69) Barnes *et al* (43) have recently fed spectroscopically active fatty acids to fasting normal and adrenal ectomized rats While they were able to identify the administered fat in the livers of their normal animals, this was not the case in the operated animals The work of Nelson *et al* (70) gives an indirect indication of the decreased catabolism of fatty acids after adrenalectomy They found that the rate of utilization of intra venously injected sodium β hydroxybutyrate was markedly reduced in adrenal ectomized rats, as compared to normal animals Since adrenalectomy does not change the blood ketone level it may be inferred that the production of ketones from fatty acids is diminished in the absence of the adrenals

It should be noted that, while the effect of the adrenal cortex *on hepatic glu* coneogenesis is unquestionable, there is as yet, little evidence that this influence is a specific one, exerted directly on the liver The fact that salt treated adrenal ectomized animals, when fed, can maintain good carbohydrate levels suggests that

the reduced carbohydrate levels of fasting may result from a disability in the mobilization of protein and fat from the peripheral stores

Finally it should be emphasized that while the separation of adrenal cortical functions into mineral and carbohydrate groups is a convenient point of view there is a certain amount of overlapping of functions Thus Anderson and Joseph (71) have shown that salt treatment has a beneficial effect upon the fasted adrenal ectomized rat both as regards increasing the survival period during the fast and in

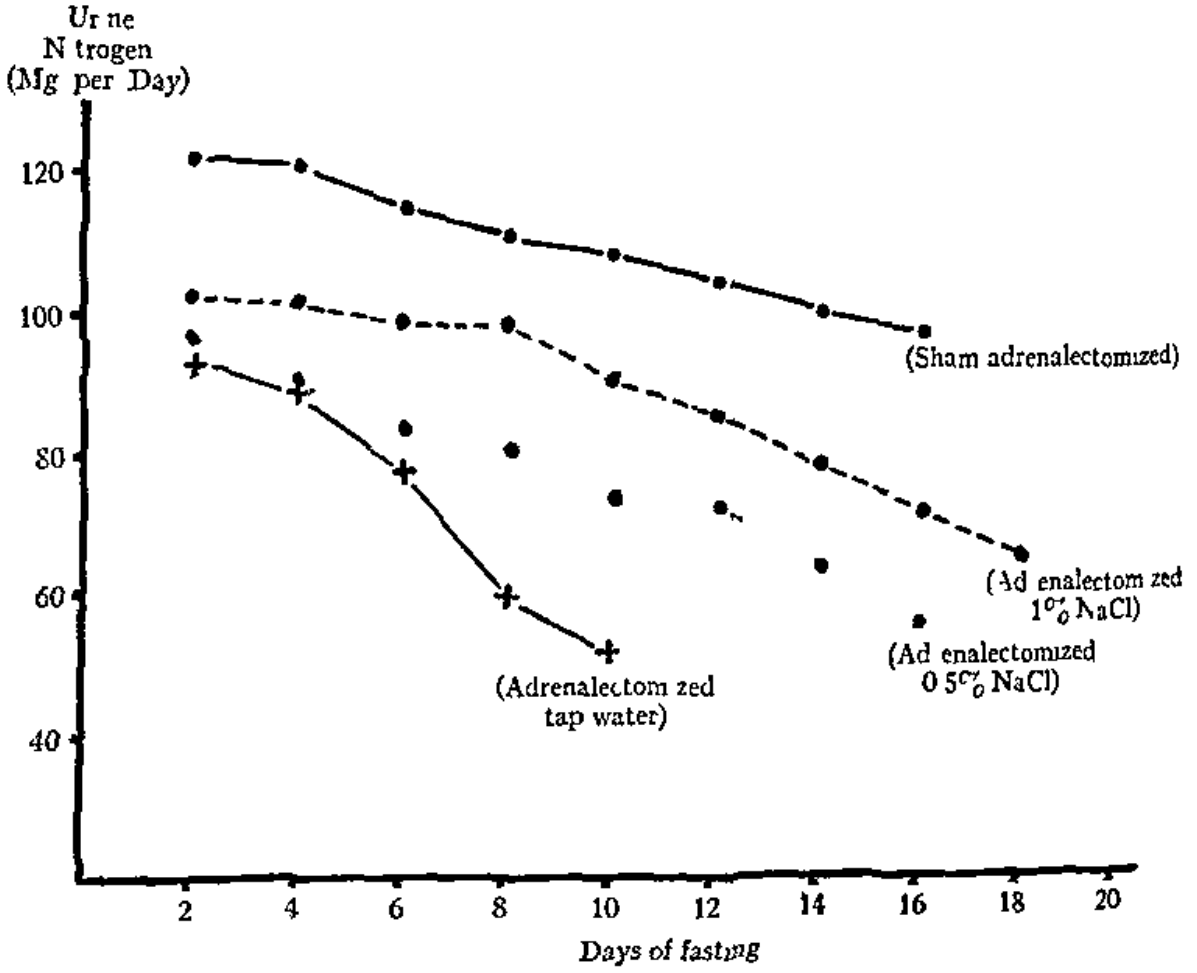


FIG. 51.—Influence of salt treatment upon the nitrogen excretion of the fasted adrenalectomized rat (From the data of Anderson and Joseph [44, 71].)

creasing the urinary nitrogen excretion Figure 51 illustrates their results and indicates that the maintenance of the mineral balance in adrenalectomized animals does support gluconeogenesis to some extent A similar slight influence of salt treatment on work performance has also been demonstrated by Ingle (72) It may well be that when all the facts are known the two sets of functions will be found to depend upon the same basic enzyme systems in the cell and that they will be seen to differ only in that each is necessary for a different stage of the reaction chain

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CHAPTER XVIII

THE THYROID

CLINICIANS have long recognized the influence of hyper or hypothyroid states on carbohydrate tolerance (1, 2) and on coexisting diabetes mellitus in humans (3, 28) In sheep Bodansky (4) found that thyroidectomy caused a decrease in the blood sugar level, while thyroxin administration raised it in normal, as well as in thyroidectomized, animals However, since thyroidectomy of the normal or depancreatized dog and cat apparently has little influence on their carbohydrate tolerance, many writers have been led to minimize the role of the thyroid in this regard (5, 6, 7) It must be pointed out that most of these authors neglected to verify the hypothyroid status of their experimental animals And since Marine (8) has demonstrated aberrant thyroid tissue in over 90 per cent

hormone was administered

METABOLIC EFFECTS OF THYROID HORMONE

1 *The blood sugar level* in hypo or hyperthyroid states is influenced by the effects of the lack or excess of hormone upon the gastro intestinal tract and the

secondary to the changes in metabolic rate, for even large increases in the latter, caused by dinitrophenol administration, have no influence on the absorption of carbohydrate The influence of the thyroid on the rate of absorption of sugar is reflected in the rise and fall of the blood sugar level which follows the ingestion of a carbohydrate meal or the oral administration of sugar solution for testing purposes In hyperthyroidism the oral dextrose tolerance curve (cf chap xxi, p 248) tends to be "diabetic" in nature, in hypothyroidism it tends to be "flat" The abnormalities are not seen when the factor of intestinal absorption is eliminated by administering the dextrose intravenously

In the post absorptive state, when the blood sugar is being supplied by the liver, the susceptibility of the latter to glycogenolytic agents or influences has a bearing

[a] This effect of thyroid is not limited to the intestinal mucosa but applies also to other epithelial structures e g kidney tubules (29)

on the blood sugar level As judged by the results of epinephrin administration, the glycogen in the liver of the hyperthyroid organism is more readily broken down than that in the normal liver The actual outcome of this state of affairs is, of course, dependent upon the amount of hepatic glycogen present, and this may lead to apparently anomalous results Thus, Abbott and Van Buskirk (11) have shown that, while the induction of mild hyperthyroidism leads to an exaggerated hyperglycemic response to epinephrin, severe hyperthyroidism, which depletes the hepatic glycogen stores, may lead either to no hyperglycemic response or even to hypoglycemia

2 *The glycogen content of tissues* other than the liver is also affected by abnormal thyroid states While lesser degrees of hyperthyroidism have little effect on muscle glycogen, Dambrosi has shown that the administration of large amounts of thy

TABLE 33

RELATION OF VITAMIN B COMPLEX SUPPLY TO THE EFFECT OF THYROID EXTRACT ON BODY WEIGHT LIVER WEIGHT, AND LIVER GLYCOGEN CONTENT (DRILL *et al* [13])

EXPERIMENTAL CONDITIONS	BODY WEIGHT		LIVER		TOTAL LIVER GLYCOGEN (Mg)	REMARKS
	Initial (Gm)	Final (Gm)	Weight (Gm)	Glycogen (per Cent)		
Control group diet+200 mg yeast	215	239	3 5	2 51	86 2	Rats of the same strain were used for this work Experimental period 47 days
Diet 200 mg yeast+100 mg thyroid	209	161	3 9	0 34	13 2	
Diet 200 mg yeast 100 mg thyroid and 1 gm yeast concentrate	199	208	5 3	2 20	116 1	

roid hormone definitely interferes with the rate of recovery of glycogen in exercised muscle (12) Hyperthyroidism also depletes the glycogen of cardiac muscle There is some parallelism between the decreased carbohydrate stores and the increased excretion of creatine in the urine These effects of the thyroid hormone are not simple in their mechanism, for a lack of the hormone does not produce the opposite results Hypothyroidism is characterized only by a moderate decrease in the glycogen content of all tissues

It has become evident recently that the amount of available vitamin B complex has a bearing upon the manifestations of hyperthyroidism (30)—so much so, indeed, that it will require further work in which the experimental animals or subjects are given ample supplies of vitamin B complex, to demonstrate the pure syndrome of hyperthyroidism uncomplicated by lack of the vitamin A glimpse of the true picture has been provided in the work of Drill and his co-workers (13), summarized in Table 33 It may be seen that an amount of yeast concentrate approxi

mately six times the maintenance dose for normal animals completely counteracted the glycogen depleting effect of a dose of thyroid which caused very significant loss of glycogen in unprotected animals It is also important to note that the extra yeast prevented loss in body weight and led to an actual increase in liver weight (13, 14)

3 *The increased protein catabolism and nitrogen excretion* accompanying hyperthyroidism or following the administration of thyroid substances has long been recognized The aggravation of clinical diabetes mellitus by hyperthyroidism and its amelioration in hypothyroid states have linked the thyroid activity on protein breakdown with gluconeogenesis from protein Sternheimer (15) has now shown that the so called "latent period between the injection of thyroxin and the first rise in oxygen consumption is not a period of inactivity Within 6 hours after the injection of a single dose of thyroxin into rats, he found a loss of liver glycogen and the beginning of a rise in liver protein These changes became more marked up to about the forty eighth hour and then showed a reversal in direction By the eighty fourth hour the liver glycogen reached a peak well above the original control level while the total nitrogen of the liver, though falling, was still above the original figures These and other observations indicated that thyroxin first causes a mobilization of protein from the peripheral tissues, and also a proliferation of the liver cells, which may be partly at the expense of the initial glycogen stores Subsequently, there is a new formation of carbohydrate from protein Gluconeogenesis from protein has also been observed by Wells *et al* (16, 17, 18) in phlorhizinized normal, adrenalectomized, and hypophysectomized rats which were treated with thyroxin or thyrotrophic hormone (Table 36, p 229)

4 In view of the evidence that thyroid hormone stimulates *gluconeogenesis*, it is difficult to understand the relatively minor or negative results as regards carbohydrate tolerance which have been obtained either by thyroidectomy of depancreatized animals or by the administration of thyroid substance to such animals In 1938 Dohan and Lukens (19) reinvestigated the effect of thyroidectomy upon [illegible] which they found modification of diabetes which follows removal of the hypophysis from the depancreatized animal However, Soskin *et al* (20) later demonstrated that the administration of thyroxin to hypophysectomized dogs maintained a normal blood sugar level through long periods of fasting and increased their urinary nitrogen excretion to that of fasting normal dogs (Figs 52 and 53) It is obvious, therefore, that the secondary atrophy of the thyroid gland probably plays an important part in the decreased endogenous protein catabolism and in the related carbohydrate disturbance of the hypophysectomized animal (see chap XIX, p 229)

The deficiency in the hypophysectomized animal which is counteracted by the

thyroid hormone does not involve the breakdown and transformation of amino acids to sugar, for ingested protein which enters the blood stream as amino acids is readily converted (chap xix, p 229) The difficulty encountered by the hypophysectomized animal during fasting must, therefore, lie in the mobilization and breakdown of the body protein to amino acids It is on this portion of nitrogen catabolism that the thyroid hormone exerts its influence This localization of the thyroid hormone effect is supported by certain data obtained in phlorhizin experiments Lusk and his co-workers (21 22) showed that fasting thyroidectomized animals excreted much less sugar and nitrogen under the influence of phlorhizin than

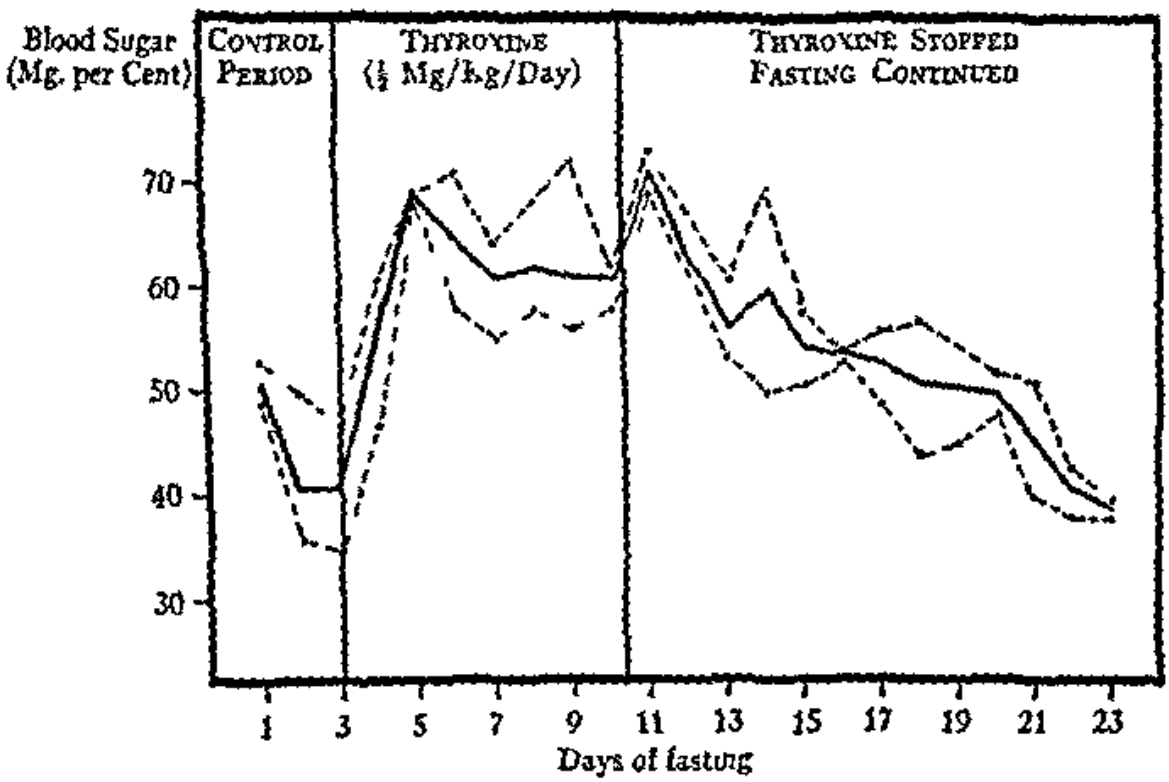

FIG 52 —Maintenance by thyroxine of a normal blood sugar level in a fasting hypophysectomized dog The upper and lower broken lines, respectively indicate the maximum and minimum blood sugar levels for each day The heavy continuous line indicates the mean value for all the blood sugar estimations (at least three per day) made on each day (Soskin *et al* [20])

did similarly treated normal animals There was no difference between the two types of animals when they were fed protein Here, again the deficiency arising from the absence of the thyroid was apparently in the mobilization and breakdown of body protein to amino acids

The question then arises as to why Dohan and Lukens, as well as previous investigators, were not able to demonstrate the role of the thyroid in depancreatized animals Indeed, they have recently reported on the subject again (23), this time to the effect that partially depancreatized cats given thyroid extract in doses sufficient to produce tachycardia and loss of weight did not exhibit any increase in gly

cosuria Anterior pituitary extract readily increased the sugar excretion in the same animals We had obtained similar (unpublished) results in our laboratory, not only in depancreatized dogs, but also in depancreatized hypophysectomized (Houssay) animals One might speculate that the thyroid influences gluconeogenesis from protein in the liver by inhibiting the previously mentioned anabolic action of insulin on protein metabolism If this were so, thyroid hormone might be expected to have little effect in the absence of the pancreas But such an action of the thyroid would be difficult to reconcile with the report of Johnston and Maroney

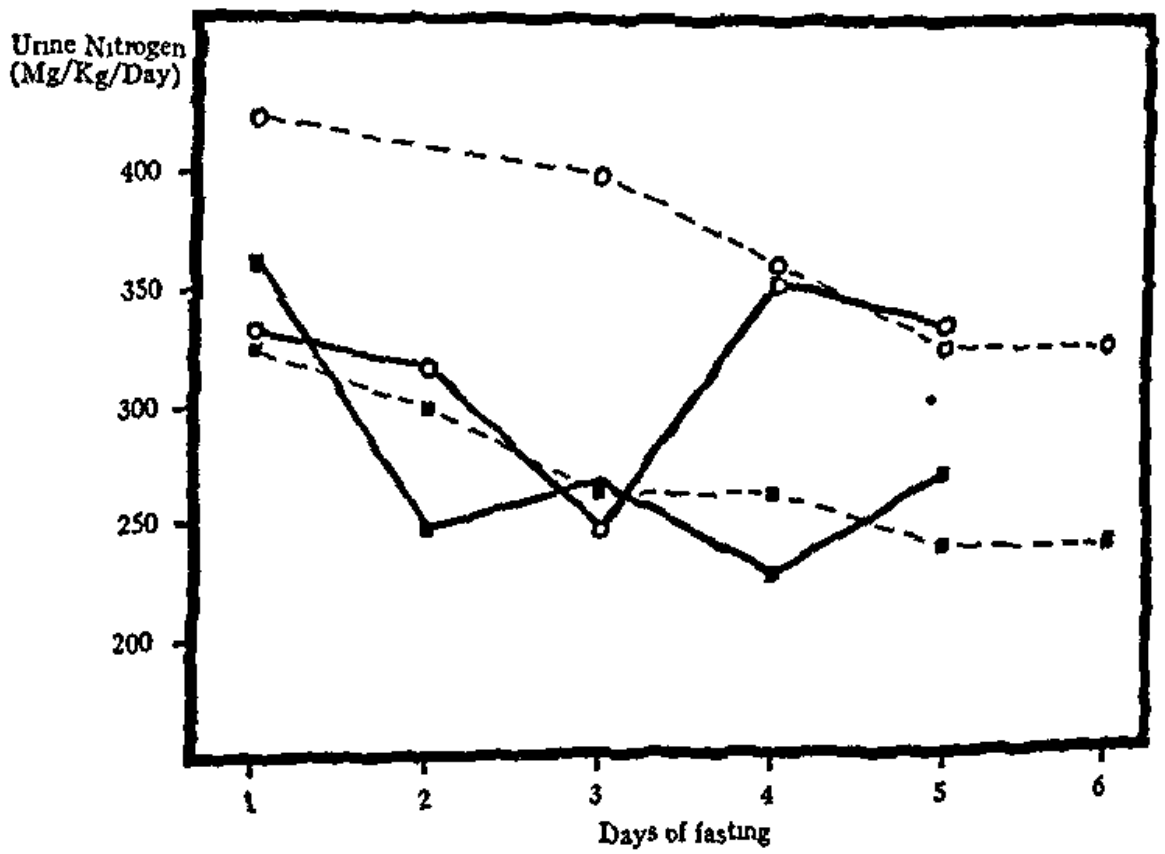

FIG 53 —Influence of thyroxine on the total urinary nitrogen excretion of hypophysectomized dogs The broken lines are taken from the figure published by Brauer (28) comparing fasting hypophysectomized to normal dogs

(24) that small amounts of thyroid are anabolic in effect as judged by the positive nitrogen balances obtained in growing children It would also be out of accord with the evidence that the growth hormone of the anterior pituitary gland is more effective in the presence of the thyroid gland than in its absence and that still greater growth can be obtained when thyroxin is administered along with the growth hormone (25) At the present time, a more likely possibility as regards the

difficulty of demonstrating the gluconeogenetic effect of the thyroid hormone in the absence of the pancreas is that the depancreatized animal given thyroid hormone may become deficient in the vitamin B complex This as was indicated in the previous section, might prevent the thyroid hormone from producing its characteristic effects

It should be noted that intensive and long continued treatment with thyroid extract can influence the severity of the diabetic syndrome by damaging the islets of Langerhans (see chap xx, p 242, "Metathyroid Diabetes)

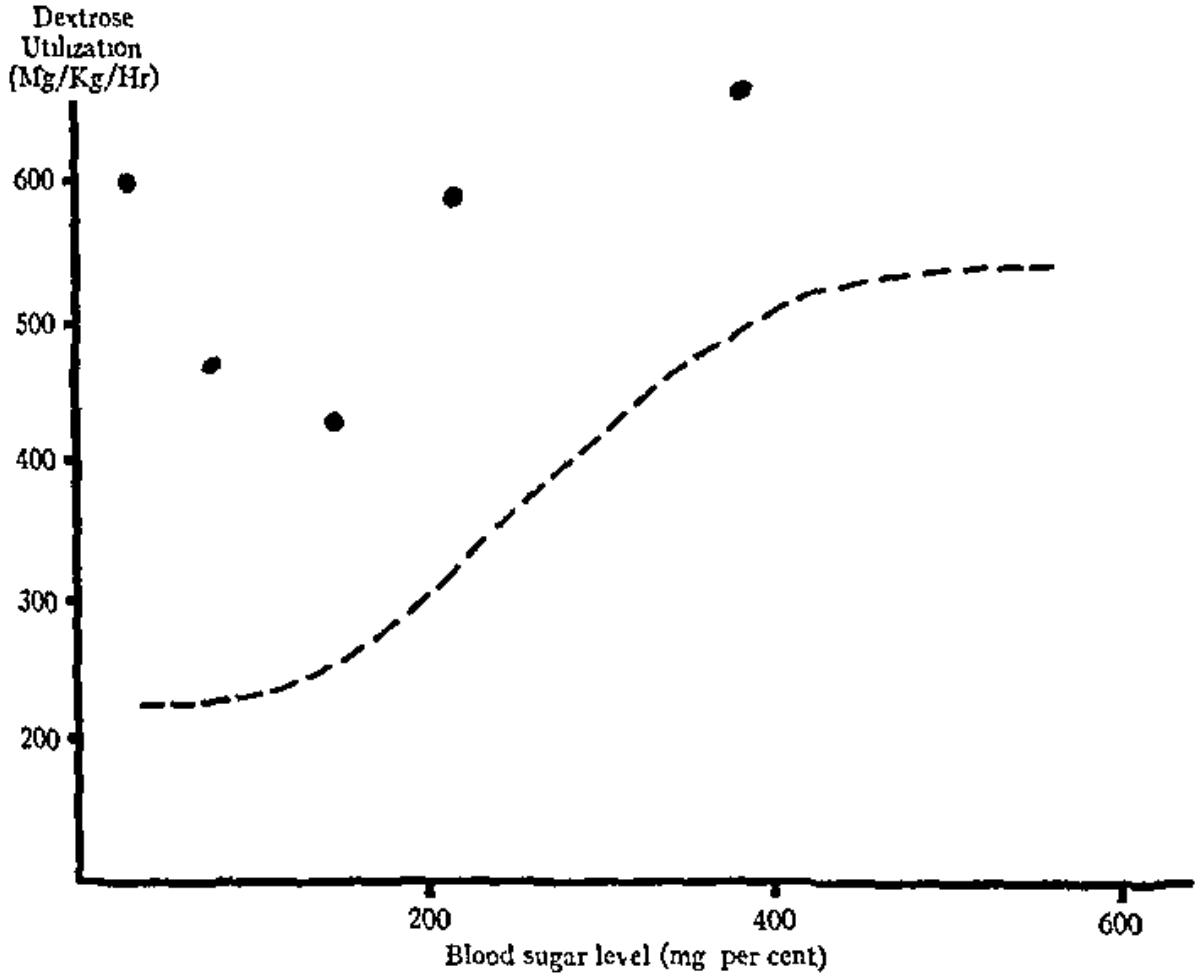

FIG 54 —The broken curve represents the utilization of dextrose by normal dogs (see chap xiv p 151) The solid dots represent the sugar utilization by dogs rendered hyperthyroid by the administration of thyroxine

5 There is an abnormally *rapid rate of carbohydrate utilization* by the peripheral tissues of hyperthyroid animals, coincident with the increased amounts of glucose entering the blood from the gastro intestinal tract and from the liver When thyroxin treated dogs are hepatectomized, the rate of fall of the blood sugar level is much greater than in hepatectomized untreated animals (26) Figure 54 compares the actual utilization of carbohydrate of normal and thyroid treated dogs as de

CHAPTER XIX

THE ANTERIOR PITUITARY

THE relationship between the pituitary gland and carbohydrate metabolism—diabetes in particular—has been known clinically for a very long time As early as 1908 Borchardt (1) recognized the large incidence of diabetes in acromegalic patients American clinicians Goetsch, Cushing, and Jacobson (2, 3, 4) wrote on this subject in 1910, and the relationship continues to be the subject of clinical writing to the present time It seems certain that, whereas the incidence of diabetes in the general population is about one half of 1 per cent, it occurs among acromegalic patients in about 25–40 per cent of cases Conversely, in hypopituitarism or Simmond's disease, hypoglycemia is often a feature, while Cushing's syndrome, with basophilic adenoma of the pituitary, is often characterized by hyperglycemia

The significance of these clinical observations has now been indicated by the work of physiologists Curiously enough, the earliest work in this direction was rather misleading, as, for example, when it was found that an extract of the posterior lobe of the pituitary gland caused a rise in blood sugar level as well as in the blood pressure More recently, however, the blood sugar raising properties of extracts of the posterior pituitary gland (Pituitrin) have been regarded as being of greater pharmacological than of physiological importance The remarkable work of the South American physiologist Houssay and of subsequent workers all over the world has shown that it is the anterior lobe of the hypophysis which is important in regard to carbohydrate metabolism

This relationship was shown by the two chief methods which are the basic procedures of endocrinologic investigation, namely, the removal of the gland, on the one hand, and the administration of extracts of the gland, on the other The effects of the removal of the anterior lobe of the pituitary gland were first shown by Houssay on toads The work was later repeated and amplified on dogs, and finally most of the effects have been adequately illustrated by Nature's own experiments on human beings

The effects of removal of the anterior lobe of the hypophysis in experimental animals or of the destruction of the gland by disease in human beings are as follows

1 *Trophic effects* —The removal of the pituitary is followed by an atrophy and decreased function of the thyroid gland (5, 6), of the adrenal cortex (7, 8), and of the gonads (9, 10), whether male or female For this reason the pituitary has often

been referred to as "the master gland" of the body However, the removal of the thyroid or the adrenal cortex or the gonads is followed by histological changes in the pituitary (11) These changes have been variously interpreted and it is still not quite certain what they mean from a functional standpoint But there can be no doubt that the removal of these other glands does affect the structure and function of the pituitary This is also true of the administration of the hormones or extracts of the other glands Thus, it is clear that, while the pituitary may be more generally important than some of the other glands, it is not merely because it dominates them It appears rather to co ordinate the functions of the other glands, so that one might call it "the executive secretary ' of the endocrine system rather than the master gland

2 *A lowering of the blood sugar level* —The blood sugar of the hypophysectomized animal under conditions of adequate nutrition is about 20–30 mg per cent lower than the blood sugar of the normal dog (1^, 13 14)

3 *The hypoglycemic effect of fasting* —A normal animal or human being may be fasted indefinitely with little or no effect on the blood sugar level As a matter of fact, there may be no significant effect until a relatively short time before death from starvation, when the blood sugar may fall precipitously However in the absence of the hypophysis, fasting is accompanied by rapid development of hypoglycemia so that the animal may die within a relatively short time in hypoglycemic convulsions (13 15 16, 17)

4 *A decreased urine nitrogen excretion* (18, 19, 20) —This is due in part to a decreased breakdown of body protein resulting from the secondary thyroid atrophy (see chap xviii, p 214) The atrophy of the adrenal cortex may also be partly responsible (see chap xvii, p 204)

5 *A decrease in the total metabolism of the body* —This is probably accounted for by the depression of thyroid activity, although other factors may be involved The other factors may be the adrenal cortical atrophy and the loss of weight brought about by the marked anorexia, which is a prominent clinical feature of pituitary insufficiency (18, 21, 22)

6 *An increased sensitivity to insulin* —A small amount of insulin which would produce no noticeable effect on a normal animal will, after the removal of the hypophysis cause prolonged and even fatal hypoglycemia (12, 23, 24)

7 *A decrease in the potassium content of the blood serum* (18, 25)

8 *A decrease in the reduced glutathione content of blood, liver, and skeletal muscle* —The diminished level of reduced glutathione in the liver may be related to the *insulin sensitivity (18, 26)*

9 *A cessation of maturation and growth* —When the pituitary is removed from immature animals, there is a cessation of maturation and growth (27, 28, 29)

The injection of crude extracts of the anterior lobe of the pituitary into hypophysectomized animals has been shown to prevent or reverse the consequences of

the removal of the gland Normal animals receiving pituitary extracts exhibit a hypertrophy and hyperfunction of the other endocrine glands (8, 10, 30) Depending upon the conditions, there may be concomitant gain in weight or increased rate of growth, or hyperglycemia, glycosuria, and ketosis may develop (31, 32, 33) Under circumstances in which there is hyperglycemia and glycosuria, there is also an increased excretion of nitrogen (31, 33) Where gain in weight or an increased rate of growth is a major consequence, there may be a retention of nitrogen (31, 32)

EXTRACTS OF THE ANTERIOR PITUITARY

The multiplicity of effects resulting from the removal of the gland or the administration of extracts led to many attempts to refine anterior pituitary preparations in such a way as to obtain products with a single or specific activity Depending upon the method of extraction or purification and upon the test animal and experimental conditions employed, a large number of different anterior pituitary factors have been claimed Collip (34) has recently listed these as follows "growth stimulating thyrotropic, gonadotropic, corticotropic, lactogenic, diabetogenic, ketogenic, liver fat increasing R Q lowering, blood lipid increasing oxygen consumption increasing, anti insulin, anti-epinephrin, glycotropic, glycostatic, and chromatophore expanding actions"

There are few who believe that these numerous effects obtained under different conditions of experimentation indicate that there are as many separate hormones secreted by the anterior hypophysis Collip suggests that as few as two or three separate hormone proteins may account for all the functional activity The dosage may play a role, since for example the growth hormone in small doses has only growth effects, while in larger doses it also exerts some corticotrophic and lactogenic action Species differences in the test animals may also be a factor Anterior pituitary extract causes a permanent diabetes in dogs but fails to do so in rats (35) There is also the probability that a number of functions listed by Collip are actually duplications of other effects Thus Jensen and Grattan (23) have reported that the anti insulin effect of anterior pituitary extracts is due to the adrenotrophic fraction They found that the administration of adrenotrophic extract, adrenal cortical extract, and corticosterone to mice resulted in a significant resistance to the action of insulin, while the injection of thyrotrophic extract, prolactin, follicle stimulating hormone and thyroxin were without effect Similarly, it has been found that the diminished absorption of glucose by the intestinal tract after hypophysectomy is probably due to a lack of the thyrotrophic hormone, for it may be corrected by treatment with thyroid hormone (36)

There are also complications of another sort in judging the demonstration of a hormone action when extracts are given or a gland is removed These complications have to do with the more or less incidental reactions of the entire organism to certain non essential materials contained in the injected gland extracts or to cer

tain secondary reactions of the organism to the condition promoted by the injec tion of a hormone or the removal of a gland Thus, Dohan and Lukens (37) have reported that the chronic administration of anterior pituitary extract to depan creatized dogs at first increased and then decreased the severity of the diabetic syndrome The serum of dogs treated for 10 months with anterior pituitary ex tract, when injected into depancreatized animals, reduced their glycosuria and urinary nitrogen excretion These results may be likened to the "anti hormone" effects previously obtained with the gonadotrophic fractions of anterior pituitary extract and, like them, are probably due to non specific antibodies formed in re sponse to the proteins contained in the injected extract

The decreased food intake which leads to marked undernutrition following hypophysectomy may also be responsible for some of the results usually attributed specifically to the lack of the pituitary hormones Mulinos and Pomerantz (38) studied the effects in rats of complete inanition during starvation and of chronic undernutrition resulting from an allowance of approximately half the normal food intake They found that the loss of weight and the histological changes in the endo crine glands resembled those following hypophysectomy The authors concluded that inanition affected the anterior hypophysis in such a manner as to reduce its secretion of the trophic hormones It would be interesting to know whether all their results would or would not have been prevented by the injection of anterior pituitary extracts into their chronically undernourished animals Levin (39) has recently shown that the decrease in weight of the viscera, which follows hypophy sectomy, can be completely prevented by force feeding the animals to the level of normal food intake Since such treatment, however does not restore the weights of the endocrine glands their atrophy is linked directly to the loss of the trophic hormones

In view of the attendant difficulties it is not surprising that the results of at tempts at the separation and purification of the various fractions of anterior pitui tary extracts continue to be difficult to harmonize and continue to disclose hitherto unsuspected effects Bergman *et al* (40) believe they have separated four entities from anterior pituitary extracts—namely, lactogenic, thyrotrophic, gonadotroph- ic, and the carbohydrate metabolism factor Meamber *et al* (41) have reported that precipitation with cysteine enabled them to separate the lactogenic and thy rotrophic effects from growth fractions of anterior pituitary extract, this procedure resulting in the preparation of almost pure growth hormone Greaves and his co workers (42) have described the properties of a more purified diabetogenic factor extracted at pH 11 It was non-dialyzable and was destroyed by a temperature of 100° C for 15 minutes at pH 10 This diabetogenic material was ketogenic and lowered the R Q It was rich in the growth factor but exhibited little prolactin ac tion

Teague (43) has reinvestigated the association of the melanophore hormone

with the "specific metabolic principle of the pituitary" previously reported by Collip and his co-workers (34, 44, 45) According to Teague, preparations of the pituitary gland rich in melanophore hormone, obtained from various sources and prepared by different methods, varied considerably in their effect on oxygen consumption in rats The melanophore activity of extracts could be selectively destroyed without removing the metabolic effects It was concluded that the melanophore hormone was not identical with a substance in the pituitary extracts which would increase the metabolic rate It was further pointed out that the results did not support the existence of a specific metabolic principle of the hypophysis, since it was found, in the course of the work, that metabolic stimulation was produced by a pituitary extract after treatment with acid and after tryptic digestion, and since such metabolic responses were occasionally obtained with extracts of muscle, liver, and kidney Collip (34, 45) has also reported the action of a pituitary extract which stimulates the "dark" cells of the adrenal medulla without affecting the chromaffin tissue The extract is active when administered by mouth The significance of this action must await enlightenment as to the function of the "dark" cells Finally, Houchin (46) has been able to decrease the alkali soluble protein components of the liver with anterior pituitary extract fractions and has suggested the existence of a protein metabolism hormone which is distinct from the lactogenic, thyrotrophic, carbohydrate metabolism, fat metabolism, and gonadotrophic hormones

Probably the best isolation of purified anterior pituitary hormones from the standpoint of methodology and their most accurate characterization from the biological standpoint are to be found in the work of Fraenkel Conrat *et al* (47)

practically all the known metabolic effects of crude extracts of anterior pituitary are accounted for, except the ketogenic There is, at present, no way of rationalizing the distribution of the various effects among the different hormonal entities, nor is it possible to say whether or not some of the effects obtained with the growth and lactogenic hormones are mediated by one or more of the endocrine glands Furthermore, the separation of practically pure entities still does not preclude the possibility that they are fragments of a single complex original hormone It is obvious that much work remains to be done in this field

THE INFLUENCE OF THE ANTERIOR PITUITARY AS A WHOLE ON VARIOUS ASPECTS OF CARBOHYDRATE METABOLISM

The well fed hypophysectomized animal maintains a significantly lower blood

sugar level as will be discussed later (chap xxi p 255) The profound influence of the anterior pituitary on the carbohydrate levels of blood and tissues is most clear ly demonstrated by observing the effects of fasting When food is withheld from the hypophysectomized organism there occurs a progressive drop in the blood sugar level terminating in hypoglycemic convulsions and death (12 13) The gly cogen content of the tissues is decreased particularly that of the liver (12 49 50) This occurs even when the pancreas and the hypophysis are both removed (12 13) and the effect of fasting is exaggerated by the administration of phlorhizin (12 15)

TABLE 34

METABOLIC ACTIONS OF PURIFIED ANTERIOR PITUITARY HORMONES

Hormone	Actions	Remarks	References
Growth (GH)	1 Nitrogen retention (in presence of adequate insulin) 2 Increase in glycosuria of partially depancreatized animals 3 Increase of muscle glycogen 4 Decrease of insulin in pancreas 5 Increase of insulin in blood 6 Decrease in liver arginase	GH and ACTH oppose each other as far as growth is concerned GH and TH act synergistically	(33 47 74-76 77)
Adrenotrophic (ACTH)	1 Increase in nitrogen excretion 2 Increase in liver arginase 3 Inhibition of insulin action 4 Increase in liver glycogen	Via adrenal cortex	(47 48 74)
Thyrotrophic (TH)	1 Increase in liver weight 2 Increase in basal metabolic rate 3 Increase in nitrogen excretion 4 Decrease in tissue NPN	Nos 1 2 and 3 via thyroid	(47 77)
Lactogenic (LH)	1 Increase of insulin in pancreas 2 Decrease of insulin in blood		(74)

These effects of fasting might be interpreted in one of two ways either (1) the anterior pituitary exerts an inhibitory influence on carbohydrate utilization by the tissues and hence hypophysectomy is followed by an excessive rate of utilization with which the capacity of the liver for gluconeogenesis cannot keep pace or (2) the gland exerts its primary influence on gluconeogenetic processes in the liver and hence its removal leads to a reduced rate of sugar formation from non-carbohydrate precursors such that the amounts of sugar necessary even for normal utilization can no longer be supplied It is clear that these alternative explanations are similar to the extent that they depend upon a disproportion between the rates of sugar formation and sugar utilization But the first explanation attributes the point of influence to the peripheral tissues while the second attributes it to the liver

At the present time the evidence that is available regarding the foregoing ex

hepatic gluconeogenesis in intact non anesthetized normal and hypophysectomized animals by means of the London cannula technic From the blood sugar contents of the inflowing and outflowing hepatic blood they estimated that the rate of sugar output from the livers of their fasting hypophysectomized dogs was only about 50 per cent of the output from the livers of fasting normal dogs The work of Wells and others (60 61) in Kendall's laboratory confirmed the defect in gluconeogenesis in hypophysectomized animals and indicated that this influence of the hypophysis was exerted partly through the adrenal cortex and partly through the thyroid gland These workers studied the urinary sugar and nitrogen excretion of normal adrenalectomized thyroidectomized and hypophysectomized rats respectively treated with phlorhizin They also included animals from which both

TABLE 35*

RELATIVE STABILITY OF MUSCLE GLYCOGEN AFTER HYPOPHYSECTOMY
(SOSKIN LEVINE AND LEHMAN [58])

CONDITION	NO OF DOGS	AV MUSCLE GLYCOGEN (MG PER CENT)		AV BLOOD LACTIC ACID (MG PER CENT		AV DECREASE IN MUSCLE GLYCOGEN (MG PER CENT PER HR)	AV INCREASE IN BLOOD LACTIC ACID (MG PER CENT PER HR)
		Initial	Final	Initial	Final		
Normal	15	511	355	50 5	106 7	43 1	15 2
Normal given anterior pituitary	13	601	448	59 0	124 6	42 5	18 2
Depancreatized	12	337	217	118 1	183 2	38 4	21 0
Hypophysectomized	5	584	570	27 3	62 8	4 1	9 5

* Changes in muscle glycogen and in blood lactic acid in live less dogs during experiments in which the blood sugar was maintained at/or above the normal level by constant injection of glucose

the thyroid and the adrenal glands had been removed By administering various hormones and combinations of hormones to the operated rats they were able to judge which hormonal factors restored the hypophysectomized animals to a normal response so far as sugar and nitrogen excretion were concerned Their results are summarized in Table 36 It may be seen that neither thyroid nor adrenal cortical hormone by itself was able to rectify the deficiency in hypophysectomized rats while the combination of both hormones was successful It may be concluded that the gluconeogenetic influence of the thyroid gland (chap xviii) and of the adrenal cortex (chap xvii) are each partly responsible for the total effect of the anterior pituitary

INFLUENCE OF THE ANTERIOR PITUITARY ON GLUCONEOGENESIS FROM PROTEIN AND FAT

Figure 56 compares the effects of exclusive fat or protein feeding and of fasting on the blood sugar level of a hypophysectomized dog It may be seen that the ani

mal has no difficulty in maintaining its blood sugar level at the expense of ingested protein It cannot maintain this level when it receives only fat It is also evident that the length of time which the animal can withstand fasting depends upon its previous feedings After a protein feeding period of 10 days it took about 12 days of fasting to reduce the blood sugar to a consistently severe hypoglycemic level, after a prolonged fasting period and a rapid recovery of the blood sugar level by the administration of protein for 1 day, a second fasting period resulted in hypoglycemia within 72 hours (13)

The most obvious explanation for the ease with which the hypophysectomized animal can restore or maintain its blood sugar level from protein placed in the intro intestinal tract at a time when it is unable to utilize adequately the much

TABLE 36

EFFECTS OF ENDOCRINE STATES AND SUBSTITUTION THERAPY ON PHLORHIZIN DIABETES IN THE RAT*

CONDITION OF ANIMALS	ENDOCRINE THERAPY	URINE SUGAR (MG PER 100 GM PER DAY)	URINE NPN (MG PER 100 GM PER DAY)	D/N	COMPARED TO THE NORMAL (=100)	
					SUGAR	NPN
Normal		621	182	3.4	100	100
Normal	Thyroxin	770	171	4.5	124	95
Normal	Thyrotrophic hormone	625	196	3.2	100	107
Hypophysectomized		148	57	2.6	24	31
Hypophysectomized	Desoxycorticosterone	323	100	3.2	52	56
Hypophysectomized	Corticosterone	449	158	2.8	72	87
Hypophysectomized	Compound E	412	170	2.4	67	94
Hypophysectomized	Compound E plus thyrotrophic hormone	625	196	3.2	100	107

* Data taken from the work of Wells and Kendall (60, 61)

larger amount of its own tissue protein for the same purpose, is the fact that in gested protein enters the blood stream as amino acid It may be concluded that the anterior pituitary exerts its influence on gluconeogenesis from protein by facilitating the conversion or the breakdown of tissue proteins to the amino acid stage However, the influence of previous protein feeding on the hypoglycemia of fasting also suggests that the anterior pituitary may control proteolytic processes within the cells but not be important for the transport and conversion of so-called 'storage' protein (78) The influence of the anterior pituitary on the breakdown of protein to amino acids is exerted—in part at least—through the thyroid gland This has been shown by experiments in which the blood sugar level of fasting hypophysectomized dogs has been maintained indefinitely by the administration of thyroxin (Fig 52, p 215) The thyroxin simultaneously restores the nitrogen excretion of these animals to that of fasting normal dogs (20)

That there is a difficulty in gluconeogenesis from the fat stores of the hypophy

sectomized animal is evident from the fact that fasting may induce a fatal hypoglycemia even though ample deposits of adipose tissue are present The influence of anterior pituitary extracts on gluconeogenesis from endogenous fat in normal animals was shown by the work *of Neufeld, Scoggan, and Stewart (62) They in* jected various anterior pituitary extracts as prepared in Collip's laboratory, into female mice and made chemical determinations of the entire carcasses of their animals They found an increase in the total glycogen content, a decrease in the amount of fatty acids present and no change in the nitrogen The inability of the hypophysectomized animal to *maintain its blood sugar at the expense of ingested* fat may depend upon the fact that this foodstuff is absorbed into the blood in the

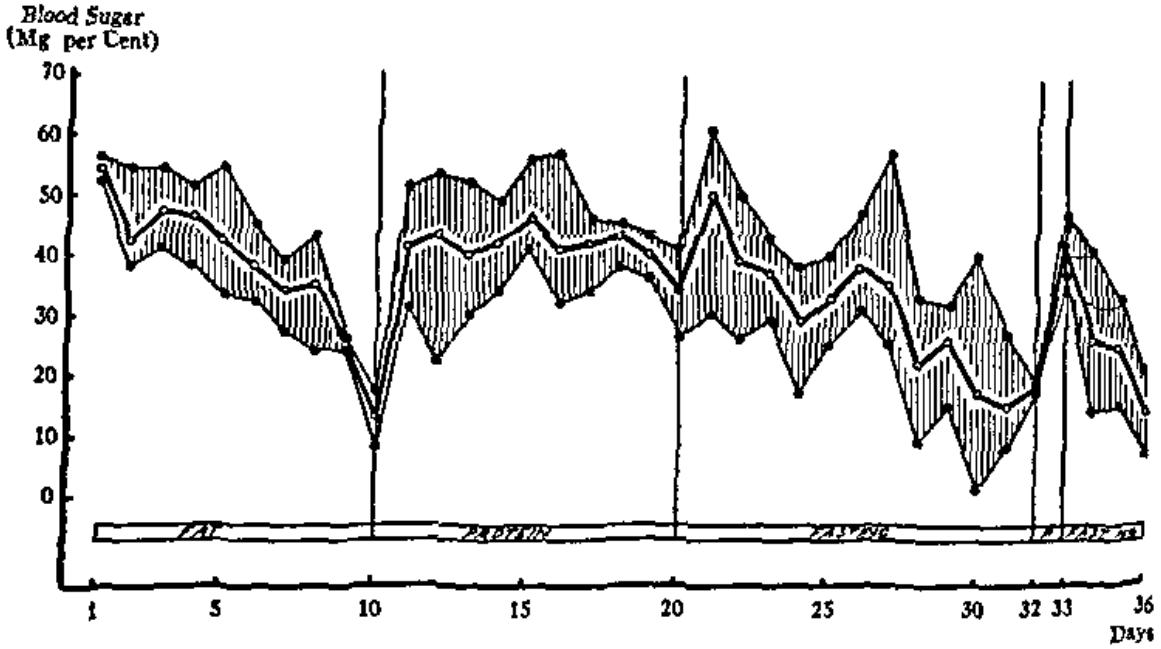

FIG 56 —Effect of exclusive fat or protein feeding and of fasting on the blood sugar level of the hypophysectomized dog The shaded area represents the spread of the blood sugar values and was obtained — — and — — — blood sugar values *for each day The central heavy* line indi nted here n of olive k n *et al*

[13])

ac no t al fat Unlike ingested hypophy

THE HYPOPHYSECTOMIZED-DEPANCREATIZED (HOUSSAY) ANIMAL

In 1930 Houssay and his associates reported their observations on hypophysectomized depancreatized dogs (12, 15) They found that such animals exhibited *less* severe diabetes than dogs with only the pancreas removed The blood sugar level varied in different animals from 320 to 113 mg per cent Sometimes spontaneous

hypoglycemia occurred The glycosuria was correspondingly variable and was en
tirely absent in some cases Nitrogen excretion was only slightly decreased but
ketosis was either very mild or absent The animals survived for months without
insulin

Figure 57 shows that fasting has the same hypoglycemic effect on the Houssay
dog as it has on the hypophysectomized animal (13) It also indicates the quantita
tive relationship between the amount of protein ingested and the consequent rise
in the blood sugar level As might be expected the glycosuria also depends upon

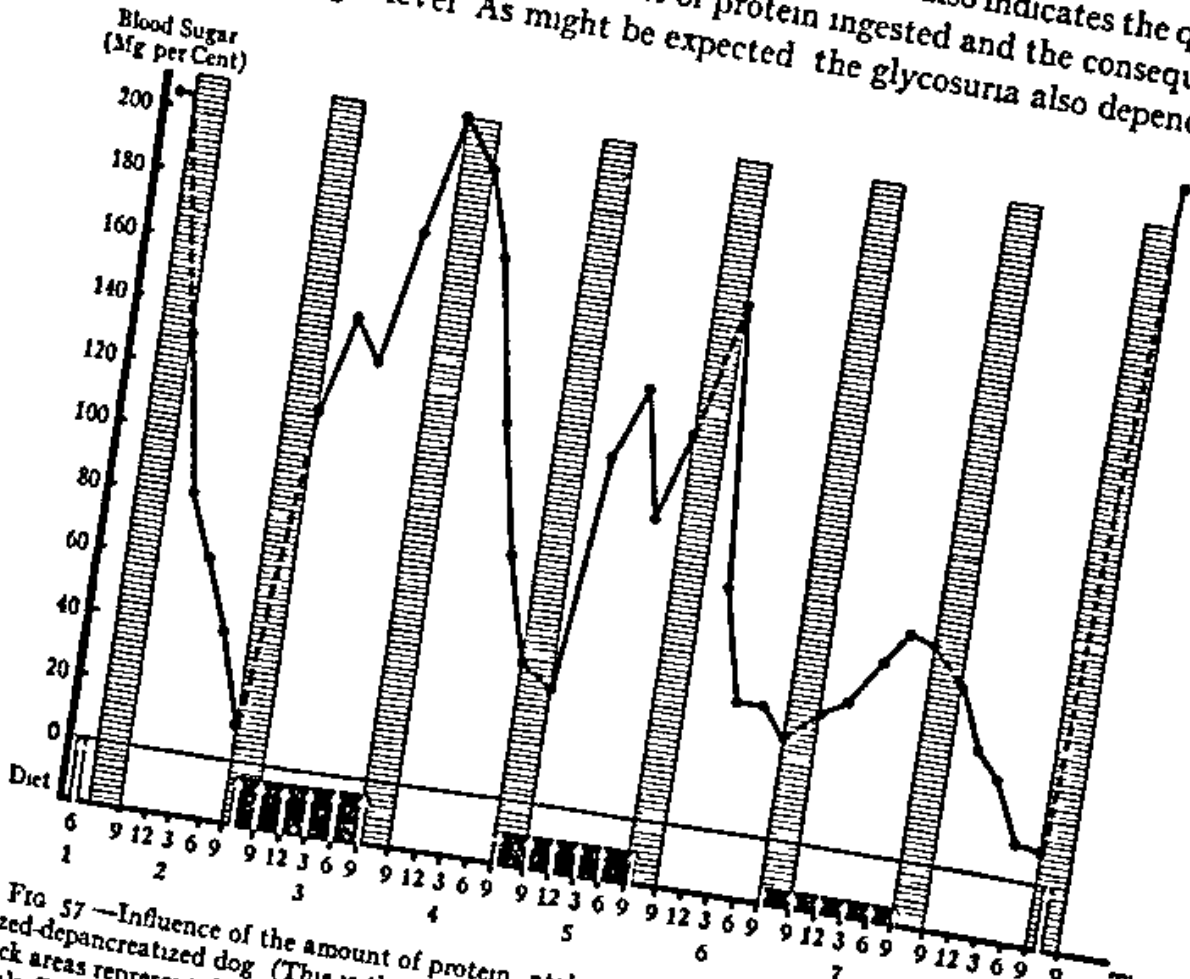

FIG 57 —Influence of the amount of protein ntake on the blood sugar level of the hypophysecto
zed-depancreatized dog (This is the same an mal as dep cted n F g 56 before pancreatectomy) The
-ck areas represent days upon which the an mal was fed the superimposed white arrows indicate the
meals The shaded strips are a foreshortened representation of the n ght periods between 9 00 P M and
9 00 A M The total amount of food given on the respective days of feeding was as follows day 1 400 gm
of lean meat 60 gm of cane sugar 120 gm of raw pancreas day 3 378 gm of protein as lean meat day 5
168 gm of prote n day 7 90 gm of prote n evening of day 8 same as day 1 (Sosk n *et al* [13])

the protein intake as is shown in Table 37 It should be noted that regardless of
the degree of diabetic manifestations in the different animals no ketonuria was
observed

It may be concluded that the same disturbance which causes the disability of
the hypophysectomized animal as regards maintenance of his blood sugar level,
namely the impairment in gluconeogenesis is also responsible for the amelora

tion of diabetes in the Houssay animal The extreme variability in the severity of the diabetic syndrome noted by Houssay and other authors undoubtedly resulted from the variability of the food intake of their experimental animals The well fed Houssay animal actually exhibits a diabetic syndrome of moderate severity, except for the lack of ketosis The undernourished Houssay animal manifests little or no diabetes But even under the most favorable nutritional conditions, the diabetic syndrome is not as intense as in the depancreatized animal with the hypophysis intact This is readily understood when one considers the unavail

TABLE 37

HYPOPHYSECTOMIZED DEPANCREATIZED DOGS (SOSKIN *et al* [13])

Dog	Survival (without Insulin) (Weeks)	Diet (400 Gm Meat 60 Gm Sugar 120 Gm Pancreas)	Ketonuria	Average Glucose Excretion (Gm per 24 Hrs)	Average Nitrogen Excretion (Gm per 24 Hrs)	*Average D N Ratio**
H 7	4	Full Partial (½)	None None	10 1 2 4	5 1 2 9	0 0
H 11	6	Full	None	80 0	14 3	1 4
H 35	7	Full Partial (½)	None None	75 0 6 1	11 7 4 9	1 28 0
H 14	9	Full Partial (½)	None None	83 0 33 5	15 9 7 0	1 50 0 50
H 30	13	Full Partial (0)	None None	70 3 0 6	12 0 1 8	0 86 0
Sally	14	Full Partial (½)	None None	61 8 39 5	15 0 14 0	0 12 0
H 4	15	Full Partial (½)	None None	95 9 77 4	16 5 12 9	2 10 2 50

* This was calculated after subtracting the amount of sugar ingested from the glucose excreted

ability of its endogenous protein and fat for gluconeogenesis and the fact that, of the ingested food materials, only sugar (as such) or protein (amino acids) can contribute to the maintenance of the blood sugar level *In other words while* the depancreatized animal with hypophysis intact can make excessive sugar at the expense of both protein and fat (endogenous or exogenous), the Houssay animal can *use only ingested protein for* this purpose This accounts for the hypoglycemic effects of fasting, in spite of ample fat stores, the low D N ratios the lack of ketosis, and the relatively long survival without insulin

The amelioration of the diabetic syndrome in the absence of the hypophysis resembles, in many respects, that seen in depancreatized dogs maintained without insulin on undernutrition diets composed solely of protein (63 64) It has been

shown that carbohydrate utilization proceeds at a normal rate in untreated pancreatic diabetes (chap xvi, p 185) and that hypophysectomy decreases carbohydrate utilization (Fig 55) Hence, neither undernutrition nor hypophysectomy can be held to ameliorate the diabetic syndrome by restoring carbohydrate utilization Undernourished depancreatized animals survive from 4 to 6 weeks and, despite the complete absence of insulin, become progressively less diabetic the longer they survive There is a progressive lowering of the D N ratio, a gradual increase in the R Q , and an increasing retention of administered sugar which has both protein sparing and antiketogenic actions These criteria of "carbohydrate oxidation" become apparent as the fat stores of the animals are depleted The difference between these animals and Houssay dogs consists in the means by which the diabetes is modified rather than in any difference in the final state which is reached The undernourished depancreatized animals suffer a gradual and incomplete loss of body fat as the period of undernutrition progresses, while the Houssay animals exhibit an acute loss of ability to utilize the ample fat stores which are present In both cases this leads to a decreased new sugar formation, so that utilization of carbohydrate is unmasked

INFLUENCE OF THE ANTERIOR PITUITARY ON SENSITIVITY TO INSULIN

The mechanism of the increased sensitivity of hypophysectomized animals to insulin is not completely understood It may depend on any or all of the following factors (*a*) a lack of counterregulatory response to hypoglycemia by the liver of the hypophysectomized animal (*b*) a decreased rate of inactivation of insulin by the blood and tissues of the hypophysectomized animal, so that the administration of a given dose of insulin might result in the presence of much larger effective quantities of the hormone, and (*c*) the absence in the hypophysectomized animal of an anti insulin factor which antagonizes the action of insulin in the extrahepatic tissues of the normal animal

The decreased rate of gluconeogenesis in the liver of the hypophysectomized animal may be a factor which limits the ability of the animal to restore its blood sugar level This agrees with the fact that adrenotrophic hormone or adrenal cortical extracts which increase hepatic gluconeogenesis also restore the normal response to insulin (23) But gluconeogenesis cannot be the only factor, because thyroxin, which resembles adrenal cortical extract in increasing gluconeogenesis, does not affect the insulin hypersensitivity of hypophysectomized animals (Fig 58)

The work of Kepinov (65) and that of Bodo (14, 66) indicate that the susceptibility of liver glycogen to breakdown by epinephrin is diminished in the absence of the hypophysis If this applies to the endogenous secretion of the adrenal medulla normally evoked by hypoglycemia, it would, of course, contribute to the effect of a given dose of insulin after hypoph[illegible]

It seems likely that the inactivation of insulin in the body is accomplished by sulphydryl compounds (67, 68) It has been shown that muscle extracts inactivate insulin *in vitro* by virtue of two components, one of which is probably reduced glutathione (GSH), while the other is the SH groups of proteins (68) The application of these facts to the intact living organism is indicated by the observation that the intravenous administration of cysteine is followed by decreased sensitivity to insulin It has also been shown that the livers of hypophysectomized rats have a significantly lower GSH content than those of normal rats (26) There is, therefore, some basis for supposing that the increased effect of insulin in the absence of

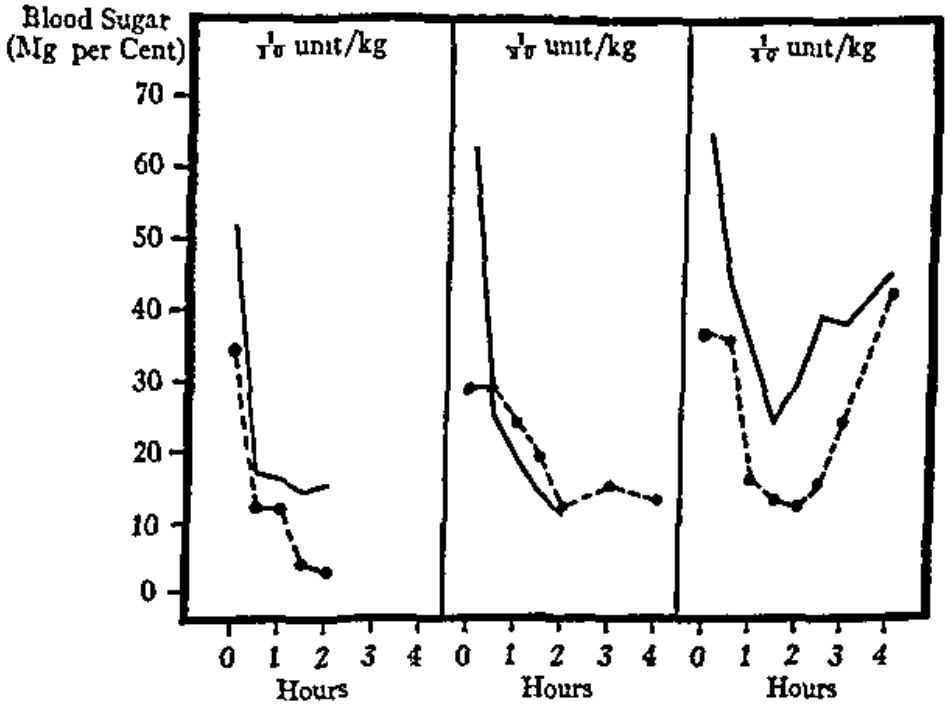

FIG 58 —Lack of influence of thyroxine on the hypersensitivity to insulin of hypophysectomized dogs Each set of curves represents a different hypophysectomized animal In each case the broken line represents the effect of insulin before thyroxine treatment while the continuous line represents the effect of the same dose of insulin during thyroxine administration Note the higher initial blood sugar values in the thyroxine treated animals (Soskin *et al* [20])

the hypophysis may be due to a prolonged period of action because of a decreased rate of inactivation

The work of Himsworth (69) may be taken to indicate the presence of a peripheral anti insulin factor in the hypophysis He reported that while the administration of crude pituitary extracts did not influence the spontaneous fall of the blood sugar in hepatectomized rabbits it did interfere with the accelerating effect of insulin upon the rate of fall The results of Russell *et al* (see p 226) appear to support Himsworth's observation But the evidence of both is opposed by the find

ings of others (p 226) which are incompatible with the conclusion that the ante
rior pituitary exerts an important peripheral action

It is evident that the sensitivity to insulin of the hypophysectomized animal de
pends—partly at least—on the liver Whether or not there is a peripheral factor
in the sensitivity must await further work The use of the more recently available
pure trophic fractions of the anterior pituitary in the liverless animal should make
a solution of this problem possible

INTERDEPENDENCE OF THE METABOLIC FACTORS

Table 34 summarizes the various known physiologic effects of the best isolated
components of anterior pituitary extract which together exert the so-called dia
betogenic action It will be noted that the most important factors are the adreno-
trophic thyrotrophic and growth hormones In general these hormones act by mo-
bilizing the non-carbohydrate precursors of blood sugar from the periphery and by
stimulating gluconeogenesis at their expense in the liver This seems an anomalous
function to attribute to the growth hormone since the process of growth must in
volve protein synthesis and nitrogen retention rather than the reverse The fact is
that the growth hormone exhibits either its anabol c or its catabolic action de
pending upon the presence or absence of insulin (33 70 71) In the normal animal
or in the depancreatized animal receiving large amounts of insulin the growth hor
mone causes nitrogen retention In the untreated diabetic animal it causes in
creased nitrogen excretion

Certain experiments showing the amelioration of the diabetic syndrome by
adrenalectomy and its exacerbation even in the hypophysectomized animal by the
adm nistration of large amounts of adrenal cortical hormone have been interpreted
as indicating that the adrenotrophic hormone is the most important factor in the
diabetogenic action of the anterior pituitary (72 73) This is not necessarily so It
is true that the presence of some adrenal cortical hormone is essential for the dia
betogenic action of the other anterior pituitary factors and this may account for
the amelioration of diabetes in its absence But it has also been shown that the
administration to an adrenalectomized an mal of an amount of adrenal cortical
hormone which by itself exerts no obvious diabetogenic effect will enable that ani
mal to yield a s gnificant diabetogenic response to anterior pituitary extracts
(50 73)

The situation is probably not so complicated as appears at present If we re
gard each of the hormones as a catalytic influence at a different point in the chain
of reactions responsible for the mobilization and catabolism of the foodstuffs it
is evident that the acceleration of any one of the reactions may increase the rate
of the whole chain However the absence of any one of the hormones may lead to
such a bottleneck at its particular point of action that the accelerating effect of any
or all of the hormones may be nullified

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CHAPTER XX

PERMANENT EXPERIMENTAL DIABETES PRODUCED WITHOUT SURGERY

THE diabetic syndrome induced in certain laboratory animals *during* the injection of anterior pituitary extracts may be termed 'hypophyseal (or pituitary) diabetes As first shown by Evans (1) and by Houssay and his co workers (2) and subsequently confirmed by many others this type of experimental diabetes begins to diminish in intensity after a few days even while the injections of extract are continued The syndrome disappears very rapidly following the cessation of treatment (3)

In 1937 Young (4) reported that the injection of increasing massive doses of crude anterior pituitary extracts into dogs resulted in some animals in a permanent diabetes which persisted indefinitely after the injections were stopped He also reported experiments in species other than the dog He found that the mouse rat and guinea pig showed hardly any effect from the injection of his crude anterior pituitary extract About half of the rabbits and rats showed slight and transitory diabetogenic effects Very young dogs or puppies resembled the rabbit and cat rather than the adult dog (5 6 7 8) Lukens and Dohan (9) were able to demonstrate the diabetogenic action of pituitary extracts and the production of permanent diabetes in partially depancreatized cats Richardson (10) made histological studies of the pancreatic glands of dogs rendered permanently diabetic with pituitary extracts and reported that the islets exhibit reduction in size, hyalinization and degranulation of the β-cells Best *et al* (11) found that the pancreas of such dogs contains from 0 to 0 2 units of insulin per gram as compared with the average figure of 3 4 units per gram in the normal animal The fact that dogs can be rendered permanently diabetic with anterior pituitary extract but that this is not possible in rats may be explained in part by the observations of Marks and Young (12) They confirmed the decrease in the pancreatic insulin content in the dog but found that the administration of anterior pituitary extract to rats increased the amount of insulin in the pancreas They reported that in this respect the rabbit behaved like the dog while the mouse resembled the rat

It is important to distinguish between the experimental diabetes seen during the injection of hypophyseal extracts (before the destruction of the islets of Langerhans and reversible) and the permanent diabetes which persists after cessation of anterior pituitary injections and which is not very different from pancreatic diabetes Hence, it seems wise to adopt the nomenclature suggested by Houssay (13)

and to reserve the term "hypophyseal (or pituitary) diabetes" for the temporary state during hypophyseal injections, while using the term "metahypophyseal diabetes" for the permanent syndrome resulting from the destruction of islet tissue

METAHYPOPHYSEAL (YOUNG'S) DIABETES

Despite their fundamental similarity, there are certain, as yet unexplained, differences between the metabolism of depancreatized dogs and that of dogs with metahypophyseal diabetes We quote Marks and Young's own summary (14) of their findings and conclusions regarding the latter type of animal These authors used the term "pituitary diabetic" to denote the metahypophyseal syndrome

1 Dogs made permanently diabetic by treatment with anterior extract differ most obviously from depancreatized dogs in the following respects
   a) Some of these dogs require more insulin for the control of glycosuria than do depancreatized dogs,
   b) The pituitary diabetic dogs are able to survive for long periods in good health without insulin therapy, if sufficient utilizable food is given The intensity of the diabetic condition may vary from animal to animal
2 Removal of the pancreas from a pituitary diabetic dog resulted in a slight and possibly not significant fall in insulin requirement The pancreas contained 2 5 units of insulin, compared with an average figure for nine normal dogs, of comparable weight, of 76 units
3 On a protein diet, the pituitary-diabetic dogs exhibited hyperglycemia, a substantial glycosuria and ketonuria, with a D/N quotient of over 3 0 in most instances, on a high carbohydrate diet, these dogs retained about 55 per cent of the total available carbohydrate in the food, on a diet of beef suet, the blood sugar level the glycosuria and ketonuria of these dogs

sulted in a substantial rise in ketonuria These results support the conclusions of Petren (1924), which were drawn from clinical investigations that protein (meat food), and not fat, is particularly concerned in the aetiology of ketonuria

4 The metabolic rate of the pituitary-diabetic dogs was somewhat above that of control normal animal under similar conditions, but the excess above normal was not so great as was found with depancreatized dogs
5 As indicated by the hypoglycemic effectiveness of 5 units of injected insulin, by the Himsworth (1936) glucose insulin test, and by the de Wesselow Griffiths (1936) serum test, the pituitary-diabetic dogs do not possess any abnormal degree of insulin insensitivity [1]

There are two additional items in their paper, not mentioned in the summary, which seem of particular interest In following up their observation of the ketogenic effect of raw meat, as compared with casein in their "pituitary-diabetic" animals, they found that the residue of raw meat which had been repeatedly extracted

[1] This statement applies only to metahypophyseal diabetes In hypophyseal diabetes there is a marked insensitivity to insulin (8)

with hot water exerted only about one-quarter of the ketogenic effect exerted by the original amount of the raw meat The supplementation of the extracted meat with a concentrate of the hot aqueous extract caused a significant increase in ketonuria Marks and Young also made a number of comparisons between their results and those obtained by Langfeldt (15) on partially depancreatized animals One might speculate as to the extent to which the differences between metahypophyseal diabetes and pancreatic diabetes might be caused by the presence, in the former, of portions of the pancreas which are not responsible for insulin secretion

The following is a partial reconstruction of the series of events leading to the development of metahypophyseal diabetes in the dog or in animals which react in a similar manner It is probable that the injection of anterior pituitary extract evokes a secretion of insulin from the pancreas Ham and Haist (16) reported an increased mitotic activity in the islet tissue of the pancreas, as well as in the thyroid parathyroid, and adrenal cortical glands following the administration of anterior pituitary extract Weinstein (17) confirmed the earlier report of Shpiner and Soskin (18) that the injection of anterior pituitary extract may cause an immediate temporary fall in the blood sugar The secretion of insulin in response to the anterior pituitary extract injection probably also accounts for the decreased nitrogen excretion (19, 20) However, the continuation of anterior pituitary extract treatment eventually exhausts the insulin secreting cells of the pancreas and apparently permanently incapacitates them (10, 11) The unopposed action of the anterior pituitary gland then becomes evident and produces an increase in protein and in fat catabolism similar to that occurring when anterior pituitary extract is injected into depancreatized animals (19)

Lukens and Dohan (9) used partially depancreatized cats with metahypophyseal diabetes to study the influence of various procedures as regards their protective action on the islands of Langerhans They found that fasting, a high fat diet, and insulin and phlorhizin administration, respectively, led to recovery from metahypophyseal diabetes, providing the treatment were started before the insulin producing cells were completely destroyed They pointed out that the obvious common factor in all these treatments was the maintenance of a lower blood sugar level over a period of time Best and his co-workers (21, 22, 23) had shown that fasting, high fat diets, and the administration of insulin diminished the insulin content of the pancreas of rats According to these workers, the histological picture of the islets of Langerhans after such treatments suggests that these procedures tend to put the β-cells of the islets "at rest" Lukens and Dohan (9) adopted this suggestion to explain their own results They concluded that in their partially depancreatized cats with limited functional reserve of the islets the administration of anterior pituitary extract led to overstimulation and exhaustion of the remaining islets through sustained hyperglycemia The various procedures which

they employed to lower the blood sugar level presumably reduced the degree of overwork of the islets and enabled them to survive and recover

While it is difficult to offer a satisfactory alternative explanation to the above there are certain obstacles to the acceptance of the postulated mechanism Thus Haist and Best reported that the insulin content of the pancreas of hypophysectomized rats was similar to that of normal rats when both types of animal were equally well fed (23 24) If the insulin content of the pancreas were a reliable index of the rate of insulin secretion by that organ, their finding would indicate that the pancreas of the hypophysectomized animal secretes as much insulin as that of the normal animal This would appear to be extremely unlikely, in view of the marked sensitivity of the hypophysectomized animal to administered insulin It therefore seems hazardous to judge the state of work or rest of the pancreas on the basis of its insulin content

As regards the influence of insulin on the histology of the islets this depends—in part at least—on the experimental conditions Mirsky (25) has shown that the continued administration of insulin to partially depancreatized dogs may actually lead to the degeneration of the pancreatic remnants! Control animals with similar amounts of pancreas removed and observed for the same length of time showed no diabetes and no evidence of any developing pancreatic insufficiency The insulin treated dogs exhibited severe acute diabetes once the insulin administration ceased and showed no tendency toward spontaneous recovery At the present time it is not possible to reconcile these results with those of Lukens and his co workers

METATHYROID DIABETES

Houssay (26) has reported that partially depancreatized dogs given large amounts of thyroid extract over a prolonged period of time eventually exhibit a permanent diabetes similar to metahypophyseal diabetes This substantiates the discussion in chapter xviii concerning the role of the thyroid in carbohydrate metabolism and enhances the probability that the anterior pituitary exerts its effects partly through this gland Houssay could not produce metathyroid diabetes in dogs with the pancreas intact

ALLOXAN DIABETES

In the course of studies on the toxic effects of alloxan (Fig 59) on the kidney, Dunn McLechie and Sheehan (27) noted (among other pathological findings at post mortem examination) a necrosis of the β-cells of the islands of Langerhans Many of their rats exhibited convulsions before death Jacobs (28) had previously reported that the administration of alloxan caused hypoglycemia in rabbits Dunn and co workers (27) confirmed this observation but found that some of their animals which survived the initial effects of the drug later developed permanent diabetes

By varying the dose of alloxan so as to avoid death from hypoglycemia and damage to tissues other than the pancreas, Bailey and Bailey (29) and Goldner and Gomori (30) were able to produce diabetes practically at will in rats, guinea pigs, rabbits, and dogs In the latter animals, kidney and liver damage seemed to be at a minimum, the acinar tissue of the pancreas and the α-cells of the islands of Langerhans appeared to be entirely unaffected, but the β-cells of the islets were completely destroyed These observations offer a new tool for the investigation of the diabetic syndrome, particularly in small animals where complete pancrea-

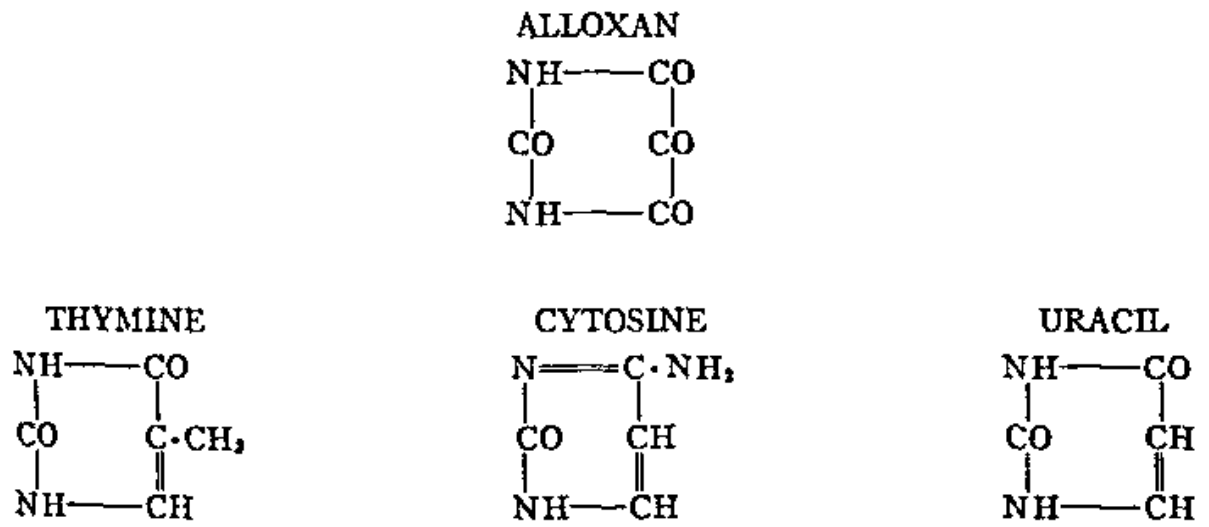

FIG 59 —Structure of alloxan, showing its close relationship to certain derivatives of naturally occurring nucleoproteins The possibility has been suggested (33) that alloxan, or a similar substance arising from a disordered nucleoprotein metabolism may have a bearing on the etiology of diabetes mellitus

tectomy has been difficult or impossible (29, 31) It may also facilitate the study of the separate functions of the component cells of the islands of Langerhans (29 32, 33)

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PART V

INTEGRATION OF PHYSIOLOGICAL AND CLINICAL ASPECTS

CHAPTER XVI

REGULATION OF CARBOHYDRATE METABOLISM

WE HAVE thus far dealt with the storage of carbohydrate its intercon versions and its utilization or dissimilation by the living organism We have seen that our knowledge of the quantitative aspects of these phe nomena is rather limited It is therefore to be expected that the development of our understanding of the mechanisms which regulate carbohydrate metabolism should be correspondingly retarded At the present time it is impossible to predict except in the most general sort of way what proportions of a given dose of carbohydrate will follow the various possible pathways for its disposal in the living or ganism under a particular set of circumstances It is impossible to calculate how much of the carbohydrate will be stored as glycogen how much will be converted and how much will be dissimilated for energetic purposes

Such partitions as might be predicted are based upon empirical data from previous experiments conducted under similar conditions We know from experience that when a limited amount of carbohydrate is available it is likely to be used as source of energy and that little of it will appear as glycogen or fat It seems obvious that there must be fairly accurate mechanisms for diverting the carbohydrate into the channel most useful for the animal but we know little or nothing of the details of such mechanisms

The regulation of the blood sugar level differs somewhat from that of other carbohydrate functions Storage interconversions and dissimilation vary with carbohydrate supply whereas the blood sugar level in the normal animal remains relatively constant under the most diverse conditions of feeding and fasting On the other hand the hyperglycemia and the great dependence of the blood sugar level of the diabetic organism on the kind and amount of ingested food indicates a profound disturbance of the regulating mechanisms in diabetes

Claude Bernard was keenly aware of the dynamic balance involved in blood sugar regulation—the balance upon which any proper conception of regulation must be based He clearly stated that the normal blood sugar level represented a precise equilibrium between the rates of sugar formation in the liver and of sugar utilization in the tissues (1) While the role which he assigned to the liver has been confirmed by most recent workers (2 3 4 5 6 7 8 9) it has nevertheless been virtually ignored in the usual explanations of the various experimental or clinical states which are characterized by a persistence of abnormal blood sugar levels Instead attention has been focused almost exclusively upon the utilization of

sugar This may be accounted for partly by the discovery of insulin and partly by the erstwhile predominance of the non utilization theory of diabetes The discovery of insulin led to overemphasis of the possible role of the pancreas in the regulation of carbohydrate metabolism, the non utilization theory demanded that the regulating activity of the pancreas be exerted upon sugar utilization

A striking example of the manner in which these factors have influenced interpretations is contained in a relatively recent review, in which an older paper by Pollak (10) is cited The latter author, by fortunate deduction from meager evidence, had arrived at the conception that the blood sugar level was a determining factor *as regards the activity of the liver* in the regulation of carbohydrate metabolism (10, 11) The quotation from Cori (12) is as follows "Pollak, before the insulin era, advocated the view that the blood sugar level is of major importance in the regulation of carbohydrate metabolism which, translated into out present terminology, means in the secretion of insulin " It will be seen, from the evidence to be reviewed, that this "translation" is not warranted and that Pollak's version happened to be more correct

THE HOMEOSTATIC MECHANISM IN THE LIVER

The characteristic rise and fall of the blood sugar following the administration of dextrose to normal animals represents a rapid and reproducible test of the regulating mechanisms Wide clinical and experimental use of this test has been made In man it has been customary to have the subject drink 300–500 cc of lemonade sweetened with 50–100 gm of dextrose The test is usually performed in the morning before breakfast, for it has been found that previous food intake influences the outcome of the test A control blood sugar determination is made before the test, and further determinations are made at various intervals up to 3 hours after the test The average, or "normal,' blood sugar curve obtained in the healthy subject is shown in Figure 60 where it is contrasted with the so called "diabetic" curve from patients with diabetes mellitus and from individuals suffering from other conditions which interfere with efficient regulation

Until a few years ago, it was customary to explain the normal dextrose tolerance curve as resulting from a stimulation of the pancreas by the administered sugar The consequent secretion of insulin was supposed to dispose of the incoming sugar by increasing the rates of storage and "oxidation" of carbohydrates (12, 13) The abnormal type of curve characteristic of the depancreatized animal and of the diabetic human was attributed to a lack of pancreatic response, with a consequent inability to dispose of the incoming sugar at the normal rate (12, 13) It will be noted that this explanation ignored the important role of the liver in supplying sugar and the possibility that regulation might also be accomplished by controlling this supply

More recently, Soskin and his co workers (14) tested the fundamental basis of

these explanations by substituting a constant injection pump for the pancreas as the source of insulin in dogs Completely depancreatized dogs received constant intravenous injections of insulin at rates just sufficient to maintain a normal constant blood sugar level in each particular animal They were therefore restored to normal in a restricted experimental sense except that they could not mobilize additional insulin, they had to get along on the constant amounts of insulin supplied

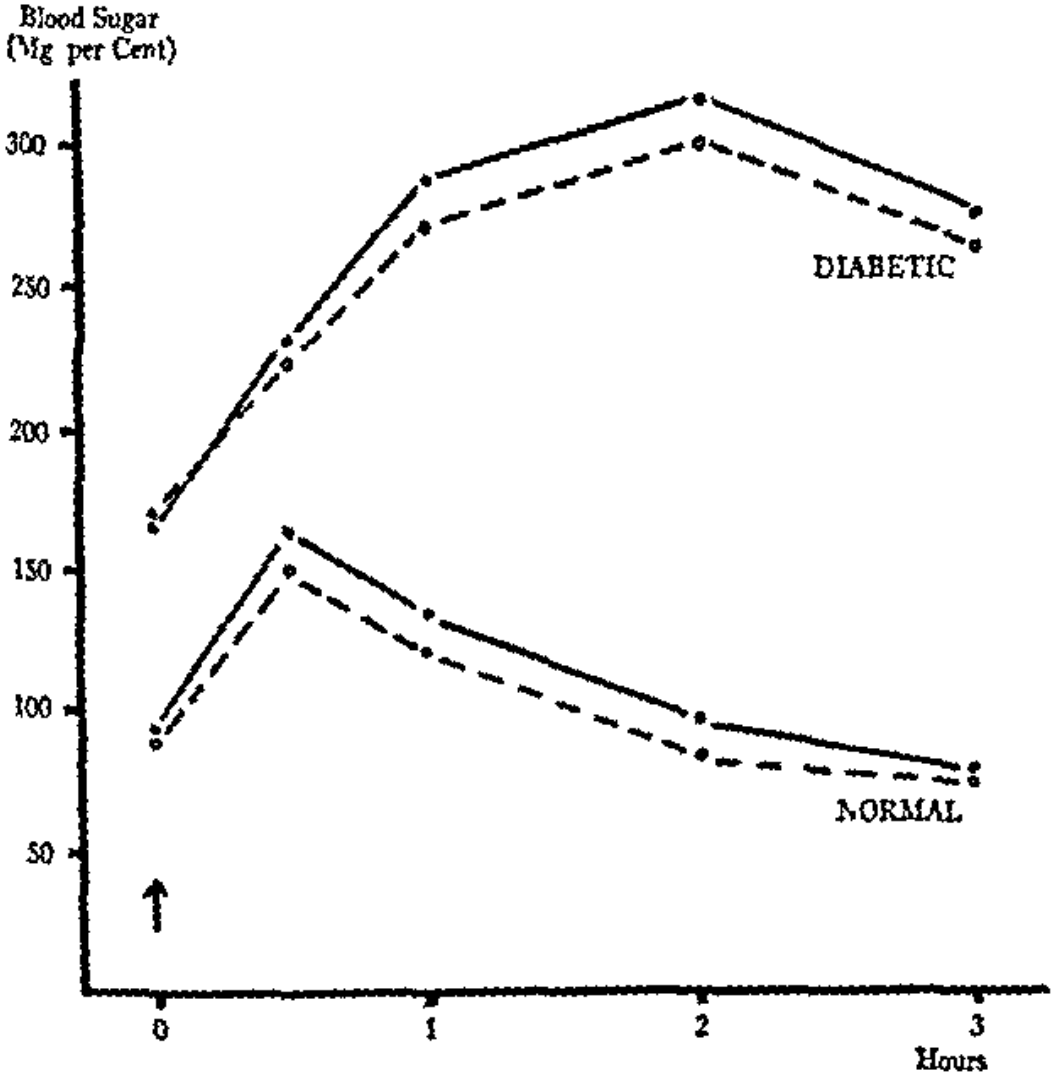


Fig 60 —Oral-dextrose tolerance curves in normal and diabetic humans The arrow indicates the administration of 50 gm of glucose by mouth The continuous lines represent arterial (capillary) blood sugar values the broken lines represent venous blood sugar values (From the data of Cavett and Seljeskog 1931)

by their artificial substitute for a pancreas If the previous concepts had been correct, such animals should have yielded "diabetic" dextrose tolerance curves But, as a matter of fact, the animals exhibited perfectly normal tolerance curves It was evident that, *provided sufficient insulin were present to maintain a constant blood sugar level* no additional secretion was necessary for adequate regulation

These results naturally directed attention toward the liver as possibly the factor that varied in regulation Normal dogs were hepatectomized, and a constant injection of dextrose just sufficient to maintain a normal, constant blood sugar level was substituted for the liver Since the pancreas was intact, this type of animal preparation was able to mobilize insulin as required but could not alter the rate at which sugar was being delivered to the blood from the artificial liver Such animals invariably yielded markedly "diabetic" tolerance curves It was apparent that the pancreas was not essential to the regulating mechanisms responsible for the normal dextrose tolerance curve, while the presence of the normal liver was essential

This led to observations on the simultaneous blood sugar values of the blood flowing into and out of the liver, in normal and depancreatized dogs, during the course of dextrose tolerance tests From these and the previous results it was postulated that (in the presence of a sufficiency of insulin, but not necessarily an extra secretion from the pancreas) the normal liver, as one of its responses to administered dextrose, decreases the output of blood sugar which it has previously been supplying from its own resources

The homeostatic regulating mechanism for the control of the blood sugar level was later subjected to direct proof (15) By correlating the rate of blood flow through the liver of experimental animals with the difference in the sugar content between the blood flowing into and out of this organ, it was possible to calculate the absolute amounts of sugar entering and leaving the liver per unit of time Figure 61 illustrates such an experiment and shows what happens when a dextrose tolerance test is made It may be seen that the liver, which was pouring sugar into the blood prior to the administration of the dextrose, ceased to do so almost immediately upon the administration of dextrose and started to take in large quantities of sugar (The period following this retention of sugar is particularly worthy of note At this time the liver neither took in nor put out sugar for a period of about an hour, showing that the inhibition of the output of sugar is a phenomenon separate from the storage of sugar) When the period of inhibition was over, the liver again began its usual supply of sugar to the blood, and the blood sugar level which had fallen somewhat below the pre test level during the inhibition rose up to and slightly above its pre-test level

In further experiments (16) it was also shown that completely depancreatized dogs which were receiving the appropriate constant injections of insulin exhibited at least as great a hypoglycemic reaction following the cessation of prolonged sugar administration as did normal dogs (see Fig 62) Like the normal dextrose tolerance curve, this phenomenon cannot be ascribed to insulin mobilization but must be accounted for by the decrease in the output of sugar by the liver in response to the influx of exogenous sugar In other words, this period of hypoglycemia following the dextrose tolerance curve or following the cessation of more prolonged dextrose injections corresponds to the time which elapses before the liver is able

to accelerate its rate of supply of blood sugar to a point sufficient to maintain the original normal blood sugar level

The hepatic regulating mechanism is analogous to the system used for the regulation of temperature in many modern homes, namely, the thermostat furnace arrangement When the temperature of the house rises above the level at which the thermostat has been set the furnace shuts off until the excess heat has been dissipated When the temperature of the house falls back to the threshold of the thermostat, the furnace starts up again That is exactly what the liver does, so far as

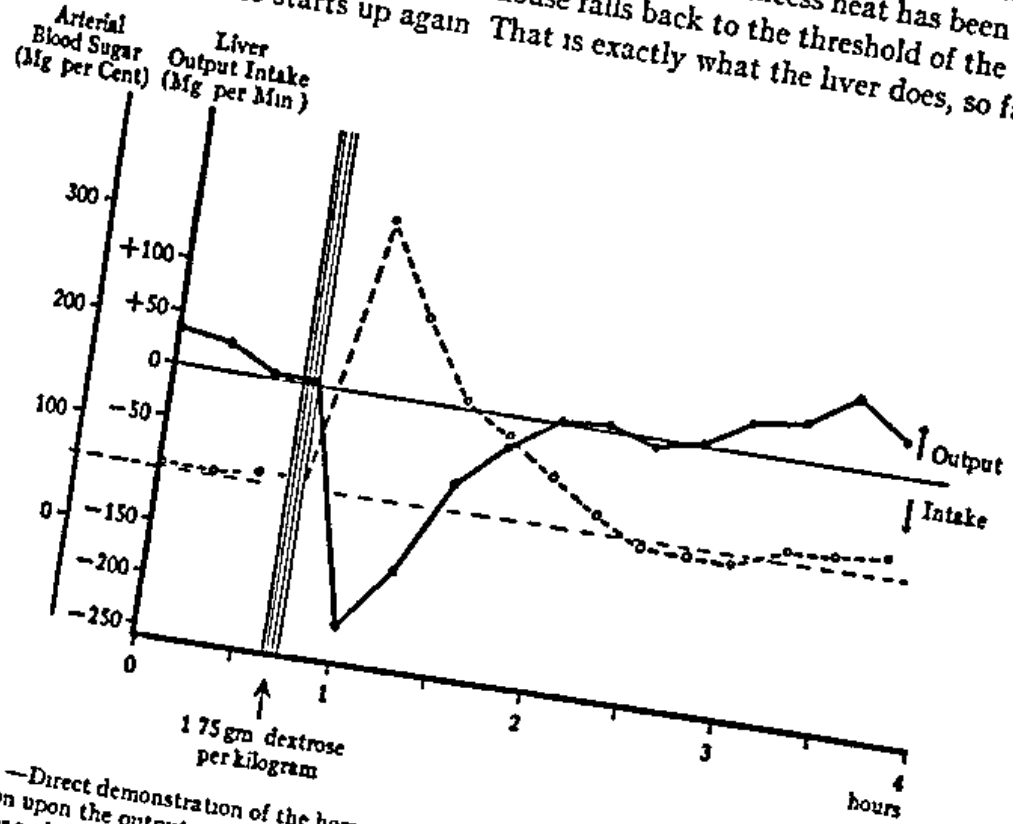


FIG 61 —Direct demonstration of the homeostatic mechanism in the liver The effect of dextrose administration upon the output and intake of sugar by the liver of an intact dog calculated from blood sugar values and thermostromuhr measurements of hepatic blood flow The broken line represents arterial blood sugar values the heavier continuous line represents output or intake of sugar by the liver in milligrams per minute Note the immediate cessation of sugar output when sugar is administered and the large intake of sugar which follows Throughout the second hour after sugar administration the liver neither retains nor excretes sugar During this period the level of sugar in the arterial blood falls below its original control values and does not return to normal until after the liver has resumed its output The inhibition of the hepatic secretion of sugar is therefore a real and separate phenomenon from the storage of sugar (Soskin *et al* [15])

the blood sugar level is concerned In this analogy the temperature is equivalent to the blood sugar level and the thermostat furnace arrangement is represented by the liver It will be noted that, just as it is the room temperature which operates the thermostat and shuts off the furnace so it is the blood sugar level which inhibits the output of sugar by the liver

Accordingly, the dextrose tolerance curve and the hypoglycemic phase which often follows it resemble the fluctuations in temperature above and below the threshold of regulation when an extra quantity of heat is introduced into the temperature regulated house The characteristics of the curve depend upon the magnitude of the disturbing factor (the amount of sugar administered), the setting and sensitivity of the thermostat (the endocrine balance), and the capacity of the furnace (the ability of the liver to produce sugar)

The fact that the hepatectomized animal with an artificially maintained normal constant blood sugar level (and with the pancreas and extrahepatic tissues free to

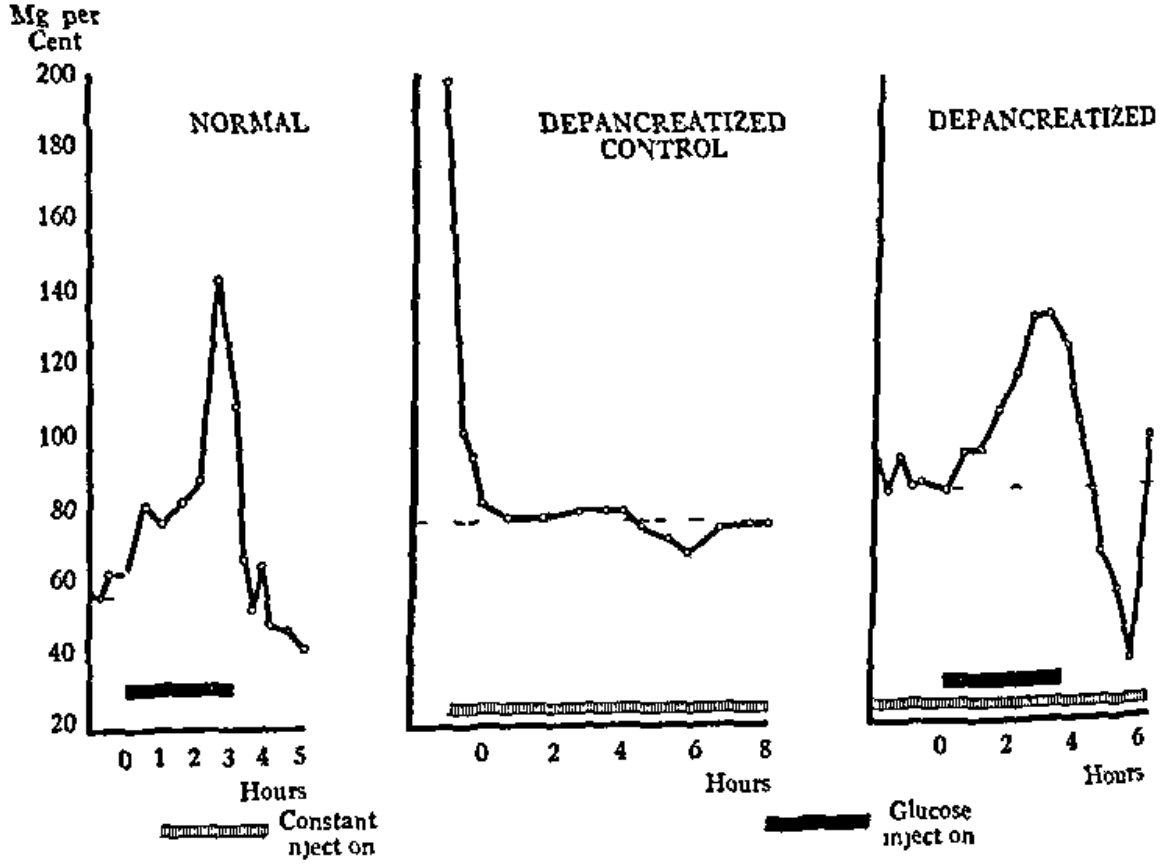


FIG 62 —Hypoglycemic reaction without extra insulin The solid black line labeled Glucose injection refers to the injection of the test sugar The crosshatched line labeled Constant injection refers to the constant injections of insulin plus dextrose that are required to maintain a normal blood sugar level

exert whatever regulating powers they possess) yields 'diabetic dextrose tolerance curves (14)' indicates the essential role of the liver in blood sugar regulation

It is not to be supposed, however, that the hepatic mechanism is the only one involved Glycogen deposition in both the liver and muscle and an increased utilization of sugar by the extrahepatic tissues undoubtedly play their parts. These processes, like hepatic homeostasis, are under the influence of the blood sugar level Cori and Cori (17) have pointed out that the rate of glycogen deposition depends upon the concentration of sugar in the blood Soskin and Levine (18) have shown that the rate of sugar utilization by the extrahepatic tissues varies directly with the height of the blood sugar level It seems logical to assume that smaller amounts of sugar, especially if they enter the circulation via the portal vein, may be fully compensated for by hepatic inhibition alone Larger amounts of sugar will invoke hepatic storage as well Still larger amounts, which, in spite of the foregoing, raise

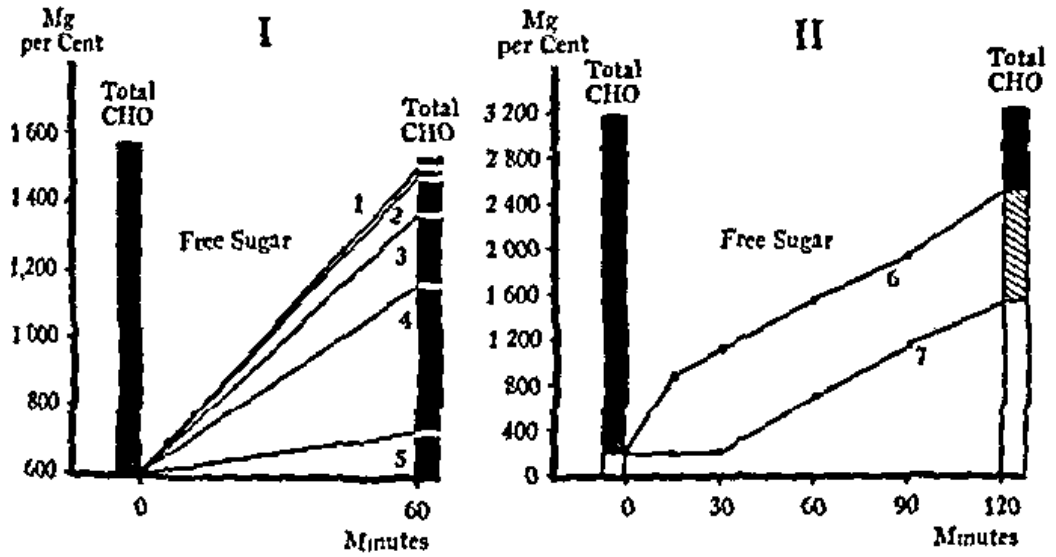


FIG 63 —Inhibition of liver glycogenolysis by added glucose

I The influence of different amounts of glucose added to each vessel upon the appearance of free sugar

The blocks representing total carbohydrate determinations at the beginning and end of each experiment indicate that there was no significant loss of carbohydrate from the system (Soskin *et al* [19])

the systemic blood sugar level, will bring into play the additional factors of extrahepatic storage and increased utilization

It is clear that the fundamental regulation of the blood sugar is an autoregulation, in which the prime mover is the blood sugar level itself This is further supported by the work of Soskin, Levine, and Taubenhaus (19) on the rate of appearance of free sugar in glycogenolyzing liver bred with and without the presence of added dextrose The results are illustrated by Figure 63 It may be seen that the sugar level influences the enzyme system concerned with the Glycogen ⇌ Glucose

(23) rather than an index of the ability to handle sugar, once it has entered the blood stream

However, even when the sugar is administered intravenously, the apparent tolerance depends upon how the data are expressed Figure 65 shows a typical intravenous dextrose tolerance curve in a hypophysectomized dog (broken line) as compared to a similar test in a normal dog (continuous line) * If the lower initial and final levels in the hypophysectomized animal are ignored, its curve appears to be low or better than normal If, on the other hand, the results of both curves are expressed as percentage rise above the initial level, the hypophysectomized curve

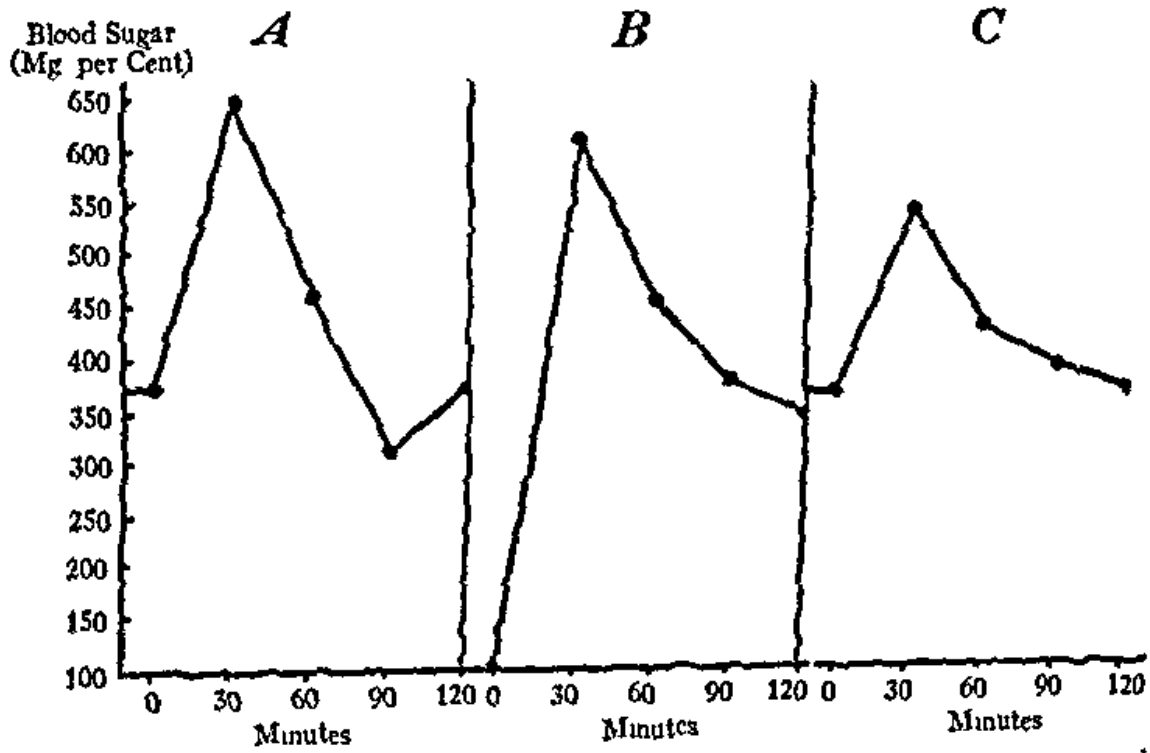
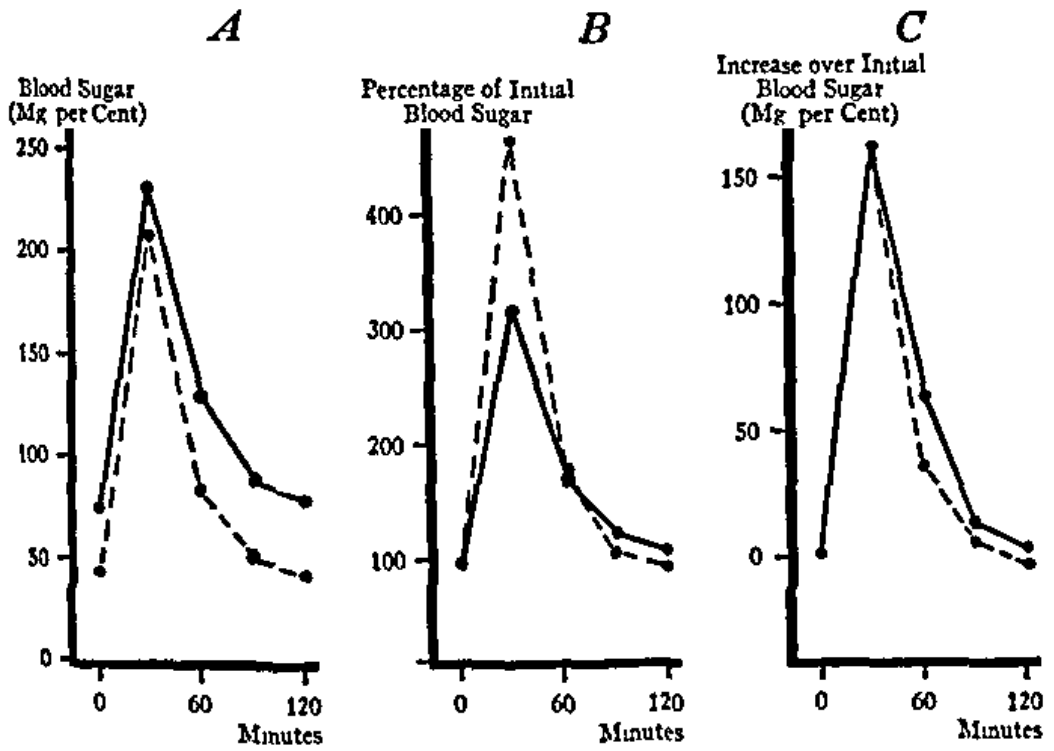


FIG 64 —Normal dextrose tolerance curves in the 'Houssay' dog Dextrose tolerance curves ob

appears to be high or worse than normal When, however, the actual curves are drawn from the same base line it can be seen that they are practically identical

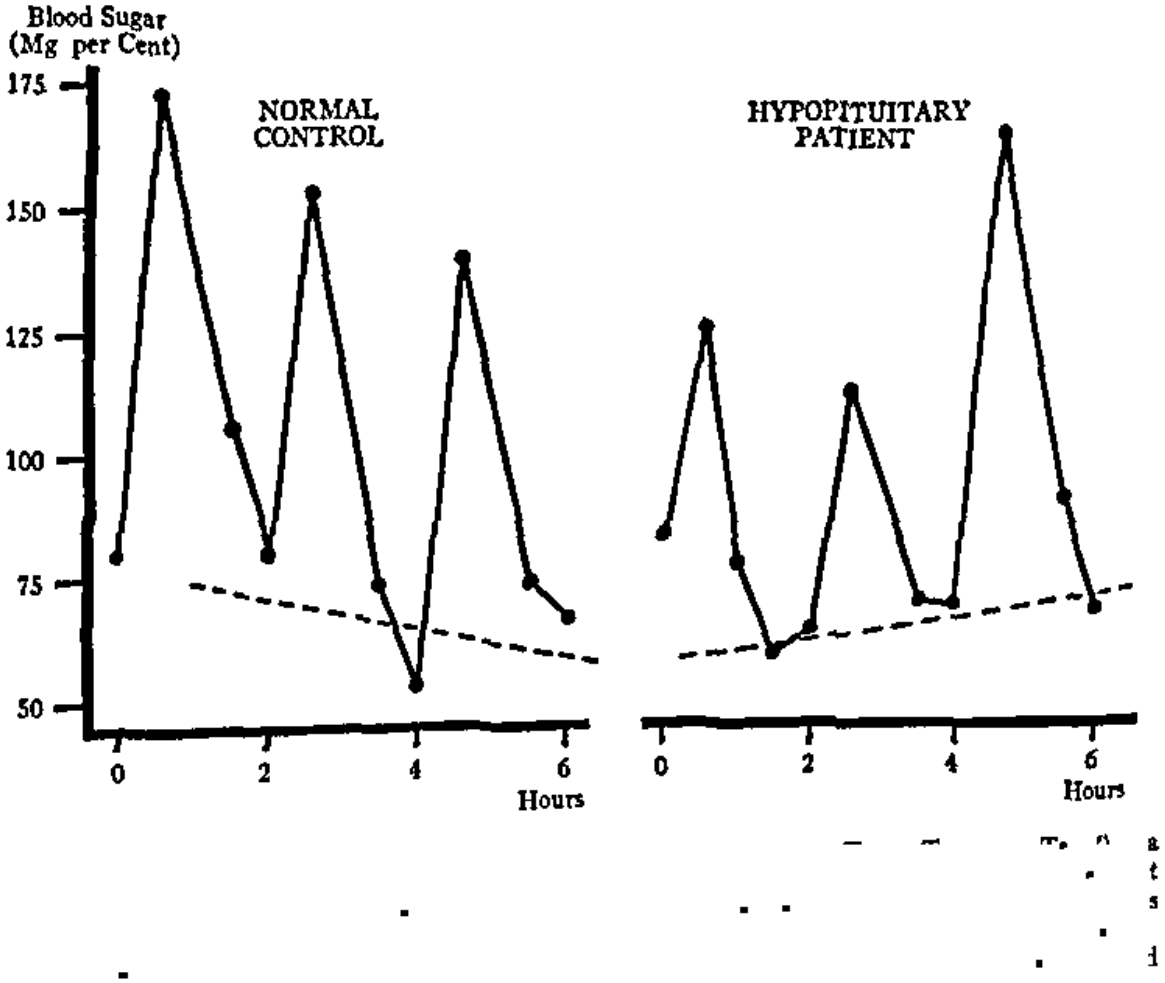
The "triple tolerance test"—Although the principal regulating action of the liver is normal in the hypophysectomized animal there is a subsidiary regulating mechanism which is not normal, namely, that mechanism which is due to the presence of the pituitary itself When dextrose tolerance tests are repeated in a normal animal, each test starting as soon as the previous one is over, it will be found that the second curve is lower or better than the first, while the third is usually better than the second The fourth curve may show some further improvement, but subsequent curves do not This phenomenon is commonly called the "Staub Traugott



effect,"[1] after the investigators who first described it (24) It has been shown that this phenomenon does not occur in the hypophysectomized animal (25)

In the absence of the hypophysis the first curve is the lowest or best one ob

tained (Fig 66) It might be supposed that this abnormality is due to some second ary effect of the absence of anterior pituitary secretion upon the function of the liver But this is not the case, for the administration of anterior pituitary extract to the hypophysectomized animal raises the level of all the tests without restoring the Staub Traugott phenomenon The lowering of the second and third curves (and sometimes the fourth) in the normal animal can therefore best be explained as a *progressive depression in the activity of the pituitary gland*, as a result of repeated or prolonged exposure to hyperglycemic levels In other words, after several suc



cessive doses of sugar the normal animal reaches that stage at which the hypoph ysectomized animal starts out This mechanism is very acceptable from the teleo logic standpoint for it is obvious that, during continued high sugar intake, regula tion will be more efficient as the threshold of the mechanism is lowered It is equiv alent to the common practice of setting down the thermostat of the house to say 50° F during the spring or fall months, when only an occasional, brief cold snap may be expected

Influence of the adrenal cortex and the thyroid gland.—At the present time it is difficult to separate the influences of the adrenal cortex and the thyroid gland from that of the anterior pituitary. Indeed, some of the influence of the anterior pituitary gland described above may be exerted through these other glands (26, 27). At any rate, deficiency or removal of the adrenal cortex, on the one hand, or the administration of potent extracts of this gland, on the other hand, will lower or raise the blood sugar level in a manner resembling that which occurs when the pituitary hormone is varied. To a lesser extent this is also true of the thyroid (28). Presumably, then, the adrenal cortex and the thyroid influence the threshold of regulation of the sugar level in the same manner as does the anterior pituitary.

INFLUENCE OF THE STATE OF THE LIVER ON THE REGULATION OF THE BLOOD SUGAR

Although we have compared the liver to a thermostat furnace arrangement, we have thus far considered only those factors which operate by affecting the thermostat part of the mechanism. However, it is obvious that, regardless of where the thermostat is set, the state of repair and the capabilities of the furnace will have an important bearing on the degree of regulation which is achieved. For example, a thermostat setting of 80° F. would have no meaning if the furnace were incapable of producing enough heat to raise the temperature of the house to that level. Another consideration is the speed with which the rate of heat production by the furnace can be increased or diminished. Unless such adjustments are rapid, there will be a considerable overswing before the correct temperature is reached. If the thermostat on a sluggish furnace clicks over at, let us say, 80° F., the temperature may rise to 90° or 100° F. before the effect of shutting off the furnace becomes evident. Finally, even with a furnace of great capacity and high efficiency, the degree of regulation will depend upon the magnitude of the environmental temperature change for which the furnace has to compensate. In other words, the usual nightly drop of 10°–20° F. in the outside temperature might produce practically no perceptible disturbance in the temperature of the house, while a sudden frost, dropping the outside temperature 40° 50° F., might result in a downward dip in the house temperature before the furnace could cope with it. The analogous considerations apply to the liver as the organ which makes the blood sugar.

An example of a disturbance in sugar regulation analogous to the situation in which the furnace is incapable of raising the temperature up to the level at which the thermostat is set is the effect of fasting on the hypophysectomized animal and on the hypopituitary human (29). The withholding of food in the latter organisms results in a progressive hypoglycemia. This does not depend upon any change in regulation, because the resumption of food intake immediately restores the previous blood sugar level. It does depend upon a marked reduction in the ability of the liver to make blood sugar from body stores, so that it cannot supply sufficient

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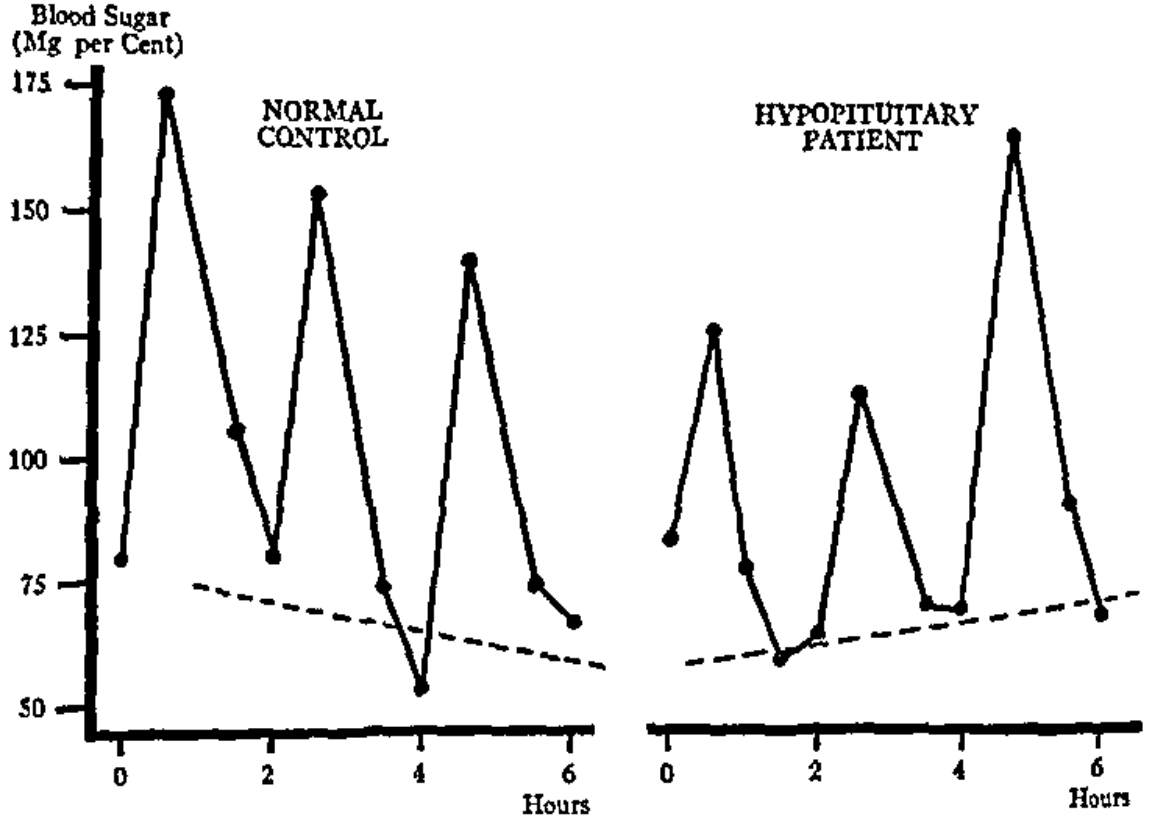

Fig 66 —Consecutive dextrose tolerance curves at 2 hour intervals (Triple Tolerance Test) in a normal human and in a proved case of hypopituitarism Twenty five grams of dextrose in 30 per cent aqueous solution was injected intravenously in every instance Note the sharp contrast between the slopes of the lowest points in each series as indicated by the broken lines This test has been found to be a use ful objective criterion in the study of proved and suspected cases of hypopituitarism in humans when used in conjunction with clin cal data (Soskin [38])

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sugar to maintain the blood sugar level unless additional preformed sugar or amino acids regularly enter from the gastro intestinal tract (27)

The situation in the liver which is analogous to the sluggish furnace, unable to increase or decrease its rates of heat production very readily, is that where the liver is damaged by toxic agents It is well known that the "diabetic' type of dextrose tolerance curve is obtained in this condition (30 31)

The "diabetic ' type of tolerance curve obtained in starvation or on a high fat diet is analogous to the temporary breakdown in the temperature regulation of the house when a sudden great demand is made upon even a very efficient furnace Both starvation and fat feeding are alike in that no preformed carbohydrate is being received by the body, so that the liver must make all the necessary carbohydrate from its own resources This represents a high degree of activity on the part of the liver, as compared to the normal conditions, under which it need manufacture only a small proportion of the body's requirements The deceleration of sugar output by the liver when sugar is administered requires a longer time when the liver is working at top speed than when it is working at half or quarter speed The essential correctness of this interpretation is supported by the fact that it is only the first dose of sugar given to a starved or fat fed animal that results in the "diabetic type of curve The second dose (by which time the liver has been able to slow up its production) usually shows a return of the dextrose tolerance curve toward the normal (32)

ACTUAL COMPLEXITY OF REGULATION IN THE LIVING ORGANISM

Thus far, the analogy of the thermostat furnace arrangement has served us well in helping to simplify the relationship between the endocrine glands and the liver in the regulation of the blood sugar But it is necessary to realize that the mechanism which has been described is integrated with a series of other regulatory processes in the body We have said, for example, that the threshold of regulation of the liver is determined by the endocrine balance But what determines the characteristic rates of activity of the endocrine glands which maintain this balance? This

that the blood sugar level affects not only the liver but also the activity of the anterior pituitary gland which in turn influences the reaction of the liver to the blood sugar level (22) There is also evidence that the concentration of sugar in the blood passing through the pancreas influences the rate of secretion of insulin (33) Furthermore, the concentration of a given hormone in the blood may have a controlling action upon the activity of the gland which secretes that hormone (34) Another mode of regulation may occur by the controlling effect of the hormone of one gland upon the rate of activity of another gland An example of the latter type of

effect is the excessive stimulation of the secretion of insulin by the repeated injection of massive doses of extracts of the anterior pituitary gland eventually leading to islet exhaustion and pancreatic diabetes as first described

It is not unusual in these [illegible] ping mechanisms all duplicate [illegible] capable of serving the function to a considerable [illegible] while other mechanisms are impaired by disease or by an experimental procedure This situation exists in regard to the regulation of the blood sugar It has been possible to demonstrate a primitive type of regulation of sugar output by the liver which can occur in isolated hepatic tissue in the test tube (19) (see p 253) In other words the output of sugar is to a certain extent controlled by the concentration of sugar present even in the absence of any possible endocrine adjustment In addition to this intrinsic hepatic mechanism and its endocrine regulators which have already been discussed there are also certain emergency mechanisms mediated by the central nervous system and the adrenal medulla (see chap xv p 168) The latter mechanisms are not evident under normal conditions and they can be entirely eliminated experimentally without appreciably affecting the sensitivity of regulation But when under abnormal conditions of stress and strain the organism is threatened by an unduly rapid or profound hypoglycemia the emergency mechanisms rapidly come into play by breaking down liver glycogen and providing the needed blood sugar

It may be helpful to think of the relationships between the emergency mechanisms the endocrine glands and the intrinsic hepatic homeostasis from the phylogenetic viewpoint The fundamental or primitive regulation may be supposed to reside in the biochemical processes of the tissue cells The endocrine glands may represent a step up the evolutionary scale by providing a more sensitive and finely adjusted regulating mechanism which renders the more highly developed organism less dependent upon its external environment The emergency mechanisms may be an additional protection against hypoglycemia for the highly specialized tissues (e g central nervous system) of the most highly developed organisms

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CHAPTER XXII

PATHOLOGICAL PHYSIOLOGY AND CLINICAL APPLICATIONS

AFTER having outlined the influences of the various endocrine glands up the process of blood sugar regulation which occurs primarily in the liv it becomes a relatively simple matter to account for the characteris clinical disturbances which accompany disease or dysfunction of the glands or the liver

CLINICAL DISTURBANCES IN THE ENDOCRINE REGULATION OF THE BLOOD SUGAR

We have seen that the experimental diabetic syndrome is primarily a di turbance *in the regulation of carbohydrate metabolism (rather than of utilization* brought about by various manipulations of the endocrine glands or their hormone But in order to avoid confusion in terminology, it is necessary to remember at th outset that diabetes mellitus, as it occurs in man, is still a clinical syndrome of un known etiology The essential and minimal characteristics of this syndrome are *persistent hyperglycemia with glycosuria*—all other effects, such as polyuria, de hydration, demineralization, loss of weight, ketosis, and coma being secondary (s In the mildest disturbances the diagnosis of diabetes mellitus often cannot b finally established until the condition has progressed in severity to the point tha stable persistent criteria develop It often happens, also, that a mild disturbanc in carbohydrate regulation is found to be accompanied by hepatic damage, hyper thyroidism, adrenal cortical tumor etc If the liver disease or the glandular dis turbance is adequately treated by medical or surgical means and the carbohydrat disturbance is thereby eliminated, it is not customary to label the transitory hy perglycemia and glycosuria as diabetes mellitus

It is readily understood that the foregoing terminology is merely a clinical con vention From the physiologic standpoint it is difficult to conceive of a disturbance like diabetes mellitus, which, in some individuals, would not be found in minimal and transitory form Nor does the presence of frank and remediable liver disease or glandular disturbance necessarily make the resulting diabetes any different from that which occurs when the etiologic disturbance cannot be detected by present clinical methods It is this physiologic point of view which must be kept in mind in considering the possible etiologic factors involved in the recognized clin- ical disturbance

Since the condition, which by clinical convention is called "diabetes mellitus," is characterized, at the present time, by the very lack of any consistent demonstrable abnormality in the endocrine glands,[1] we must perforce base our notions as to possible etiology upon the various experimental procedures by which a similar syndrome can be produced These possibilities have already been indicated in the sections devoted to the various endocrine glands and the liver Their relationships to each other are graphically illustrated in Figure 67 In the balance of forces rep-

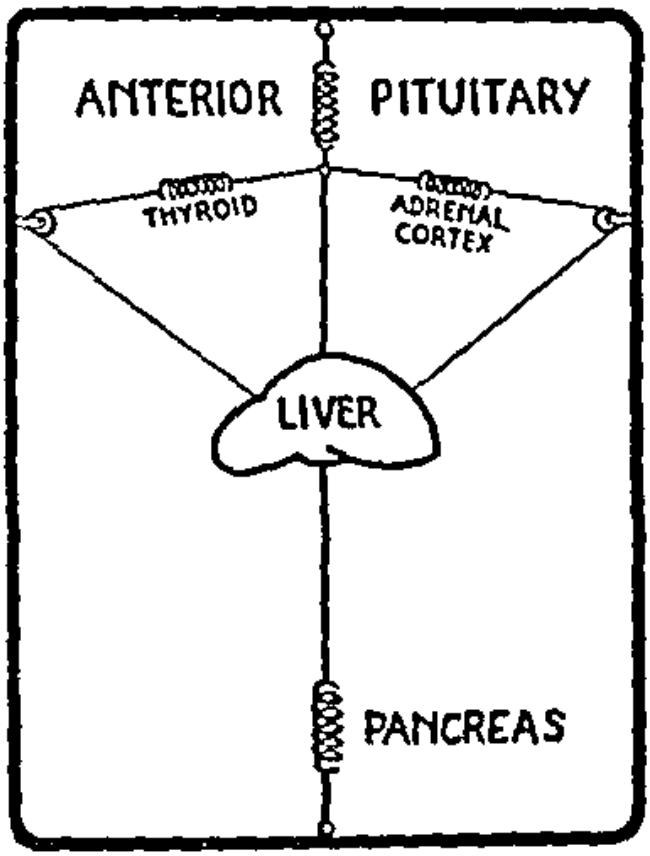


FIG 67.—Mechanical analogy to the endocrine balance

resented there, it may readily be seen that the same end result might be obtained in a variety of ways A shift of regulation toward hyperglycemia might be due to a diminution in the insulin factor (an absolute lack of insulin) or to an intensifica

[1] Two recent publications require some comment

[1] Sussman (108) has reported camera lucida measurements of the relative areas of the islets of Langerhans in histologic sections of pancreatic glands from human beings with and without diabetes mellitus. According to him the islets of the diabetic individuals occupied 0 17–4 6 per cent of the total area, as

tion of the opposing factors (a relative lack of insulin) If the latter type of disturbance is, indeed, responsible for some cases of *diabetes mellitus*, it is possible that we may eventually learn to distinguish a pituitary diabetes, an adrenal cortical diabetes and a thyroid diabetes, as well as a pancreatic diabetes To this list must be added a possible hepatic diabetes which might occur in the absence of endocrine disturbance when the liver is no longer responding normally to its endocrine regulation *It must be emphasized that none of these considerations minimizes the importance of insulin in therapy or suggests that any other efficacious agent is known at the present time* The diagram clearly indicates that the important thing from the therapeutic standpoint, is the maintenance of the normal balance The administration of insulin will correct the imbalance whether it is due to an absolute or to a relative lack of this hormone

The differentiation of the various possible types of diabetes mellitus must await the development of adequate methods for the quantitative estimation of glandular function or of the titer of the various hormones in the blood For the present all diabetic manifestations which are accompanied by a clinically recognizable dysfunction of some gland or of the liver are considered to be part of the syndrome associated with that clinical state A similar situation exists as regards carbohydrate disturbances in the direction of hypoglycemia and the differentiation between hyperinsulinism and other conditions which may lead to hypoglycemia An inspection of the following list, in conjunction with an examination of Figure 67 will relate the characteristic blood sugar disturbances accompanying the various known endocrine syndromes with the physiologic considerations which have been outlined We have included key references to articles dealing with the carbohydrate disturbance in the clinical syndrome

ENDOCRINE HYPERGLYCEMIAS

Anterior pituitary	Acromegaly (2) Pituitary basophilism (3 4)
Thyroid	Hyperthyroidism (5)
Adrenal cortex	Hyperadrenocorticalism (6 7)
Adrenal medulla	Pheochromocytoma (8)
Pancreas	Diabetes mellitus in those cases where there is evidence of destruction of the islets of Langerhans (9)

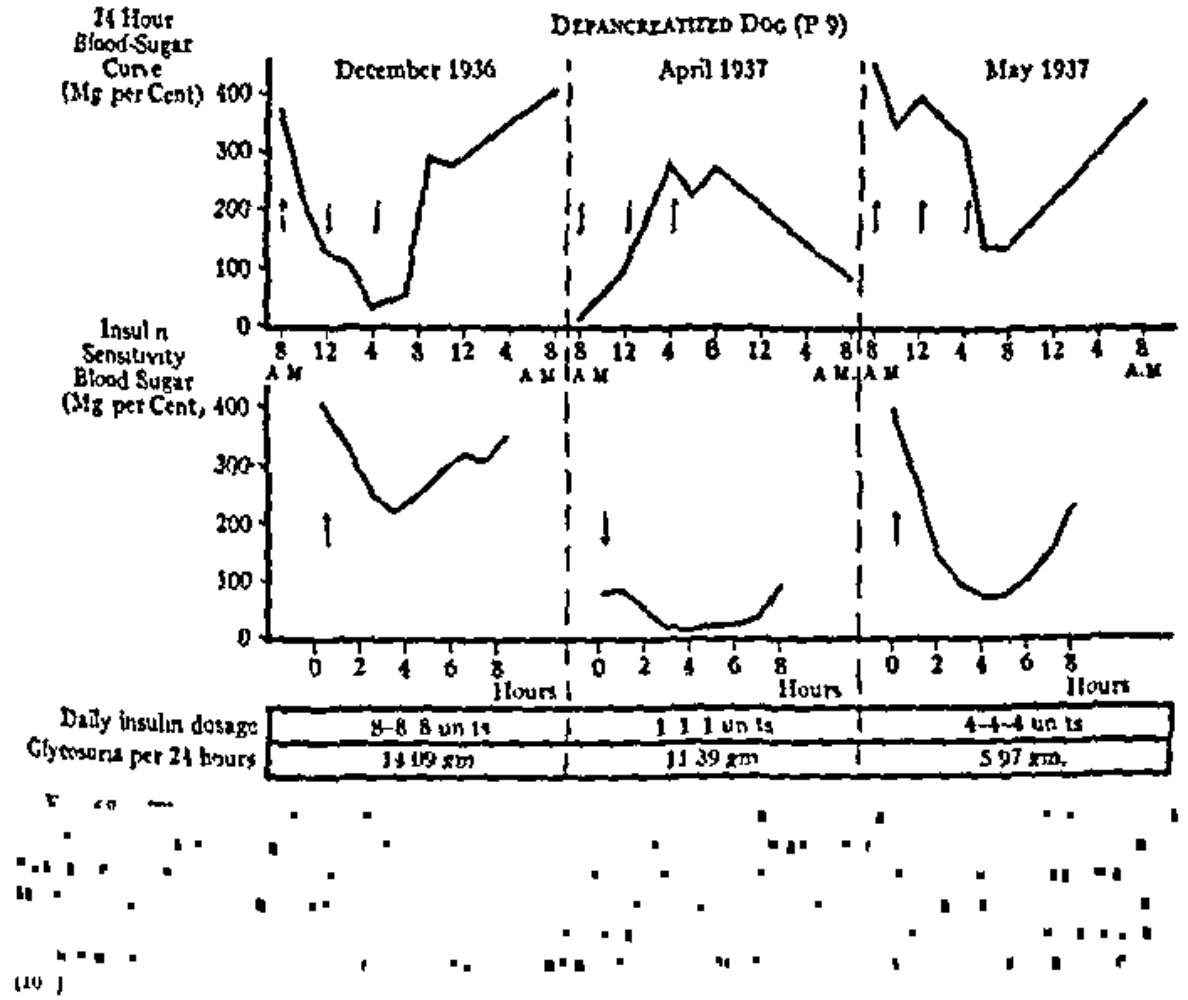
compared to 0 7 5 5 per cent in the pancreas of normal individuals Aside from the considerable overlap in these figures it should be pointed out that there is a difference of only 56 per cent in the mean values [illegible] that about 90 per cent of the pancreas

ENDOCRINE HYPOGLYCEMIAS

Anterior pituitary	Simmonds' disease (10) Anorexia nervosa (11)
Thyroid	Hypothyroidism (12)
Adrenal cortex	Addison's disease (13) Adrenal apoplexy (14)
Pancreas	Hyperinsulinism (15)

INFLUENCE OF LIVER DYSFUNCTION ON BLOOD SUGAR REGULATION

In chapter xxi (p 259) we described the various ways in which the state of the liver might affect the regulation of the blood sugar level This may not be of great



practical importance when one is dealing clinically with a case of frank liver disease, where the danger to life from other consequences of liver failure overshadows the carbohydrate disturbance But it may be of considerable value in diagnosis and prognosis when an endocrine disturbance in blood sugar regulation is complicated by the presence of liver dysfunction Figure 68 illustrates a striking example of this situation Here we have a pure endocrine disorder, namely, diabetes re

sulting from the removal of the pancreas in the dog, experimentally complicated by a reversible type of liver damage (16) It will be seen that the characteristics of the diabetes in this dog were markedly changed during the time that the liver was affected (April, 1937)

Interest in these results is enhanced by the fact that in clinical diabetes mellitus we find two similar types of the disease—namely, the insulin sensitive ("juvenile" or unstable) and the insulin insensitive ("adult" or stable) The depancreatized dog with an unimpaired liver (December, 1936) resembles the individual with insulin sensitive, juvenile, or unstable diabetes mellitus The morning fasting blood sugar is the highest in the 24 hours, the blood sugar falls sharply during the day under the influence of a dose of insulin with each meal and then rises throughout the night hours In this state the administration of 0 3 units of insulin per kilogram of body weight causes a smart fall in the blood sugar level of about 200 mg per cent

The same animal, which had been on a diet of lean meat, sugar, and raw pancreas, was then placed on an equicaloric diet from which the pancreas was omitted This is known to result in a severe fatty infiltration of the liver (17, 18) (see chap viii, p 91) The impairment of liver function consequent to the fatty infiltration is reflected in three ways which are characteristic of the insulin insensitive adult [illegible] (April, 1937) At this time the diabetes is milder

of the food intake, despite the insulin administered with the meals The blood sugar then falls during the night hours The administration of the same amount of insulin as in the previous sensitivity test now results in a much smaller drop in the blood sugar level The restoration of raw pancreas to the diet of this animal, with a return of the liver function almost to normal (May, 1937), completely reverses the nature of the diabetes to its original condition

This demonstration of the influence of fatty infiltration of the liver on the nature and severity of diabetic manifestations suggests an explanation for the partial success of the extreme high fat diets and starvation regimens formerly used in the treatment of diabetes mellitus Both these procedures will lead to a fatty infiltration of the liver It should be noted, however, that the diabetes is controlled only at the expense of liver function Hence it may be said that "the diabetes is better but the patient is worse The lack of general well being of patients under those treatments, as compared to patients under modern treatment, may well be ascribed to the difference in the functional state of the liver (19)

Toxemic liver damage—Abnormal dextrose tolerance curves have been described as occurring in patients suffering from acute infectious diseases (20) Similar disturbances in carbohydrate metabolism have been demonstrated in experimentally induced toxemias in animals (21, 22) The "diabetic" type of dextrose

tolerance curve obtained under these circumstances has been interpreted by some as being due to a lack of endogenous insulin, consequent to the functional impairment of the islands of Langerhans (20) Others have ascribed the phenomenon to an interference with the action of the available insulin, whether of endogenous or of exogenous origin (23)

Using methods similar to those which they employed in demonstrating the homeostatic mechanism for blood sugar regulation (chap xxi, p 248), Soskin and his co-workers (24) showed that toxemia affects carbohydrate metabolism by damaging the liver and interfering with its regulating mechanism Completely depancreatized dogs receiving a constant injection of insulin sufficient to maintain a con-

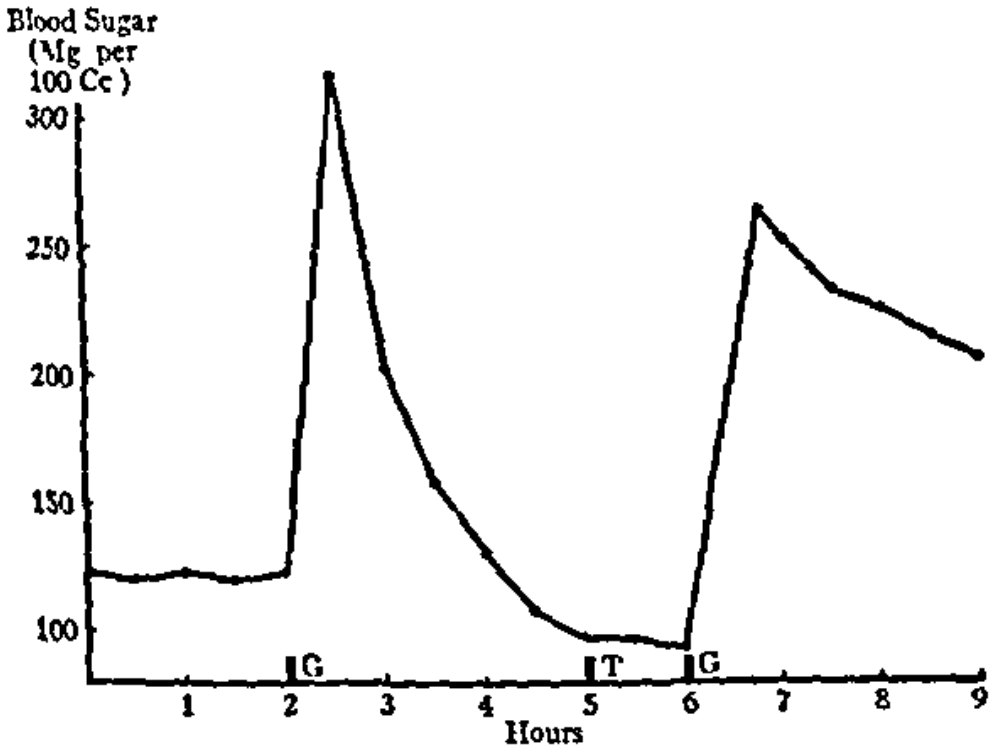


FIG 69 — Diabetic tolerance curve resulting from toxin in absence of pancreas The dog received throughout the experiment a constant injection of dextrose plus insulin just sufficient to maintain the blood sugar at a constant level *G* indicates the administration of the test sugar and *T* the administration of the toxin (Soskin *et al* [24])

stant normal blood sugar level were rendered toxemic by the intravenous administration of diphtheria toxin Figure 69 shows that such animals exhibit normal dextrose-tolerance curves before, and "diabetic" curves after, toxin administration Hence the abnormal tolerance curves cannot be ascribed to an effect of the toxin on the pancreas There is also direct *in vitro* evidence of the influence of toxins on carbohydrate metabolism in the liver (25)

Although the "diabetic" type of dextrose tolerance curve is usually obtained in toxemic states, Althausen and others (26) have shown that in less acute toxemias, where there was a longer survival period, the "diabetic" type of curve may give way to the "supernormal" before death intervenes Clinically this variation in the

abnormal curve caused by liver damage has been described by Judd *et al* (27), and it is well known that "diabetic," "supernormal," and even "normal dextrose tolerance curves may be obtained in cases of liver injury without apparent relation to the degree of liver damage as judged by clinical or pathologic criteria Indeed this lack of correlation has been reported by Mann (28) as also applying to other tests of liver function However the foregoing variations in response are not as haphazard as they appear but depend upon the stage or degree of liver damage which exists at the time the test is performed

When a slowly progressive toxemia is induced in experimental animals and tolerance curves are repeated consecutively to the point of death (29), a definite and predictable sequence of tests is obtained, as shown in Figure 70 The first effect of the toxin is to cause a "diabetic" type of curve As the toxemia progresses there is a reversal of effect, so that the curves appear to be more and more "normal " As death approaches, there is a sudden change back to the "diabetic ' type of response The sequence of events portrayed in Figure 70 was obtained when 0 9 gm of dextrose per kilogram of body weight, administered intravenously, was used as the test dose of sugar The significance of the responses becomes apparent only when they are compared with those obtained using smaller and larger test doses When this is done, it becomes evident that the "diabetic" curves obtained in early toxemia are due to an impairment of the responsiveness of the hepatic homeostatic mechanism, for at this stage a small test dose of sugar (0 25 gm/kg) yields an earlier and more "diabetic" response than a large test dose (1 75 gm/kg) On the other hand, the "diabetic' type of curve obtained in late toxemia has little relationship to the homeostatic mechanism but may rather be ascribed to advanced liver failure At this stage the animal responds to the dextrose tolerance test in a manner similar to that of the hepatectomized animal (chap xxi, p 252) The small test dose of sugar yields normal appearing curves, while the larger test doses give progressively more "diabetic" curves

Figure 71 diagrammatically summarizes the progressive change in liver response to administered sugar This may be explained on the basis that the first effect of a poison on the liver is to act as an irritant to the glycogenolytic mecha

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rise in blood sugar would inhibit this process As the effects of the toxin on the liver progress to the point of mortal damage to the hepatic cells, the latter must pass from the stage of glycogenolytic hyperirritability, through normal irritability, to hypo-irritability and death Translated into terms of the inhibitory reaction which determines the character of the dextrose tolerance curve, this cycle of events would be (1) a decreased inhibition of glycogenolysis yielding "diabetic' tolerance curves, unless the strength of the stimulus, as represented by the admin

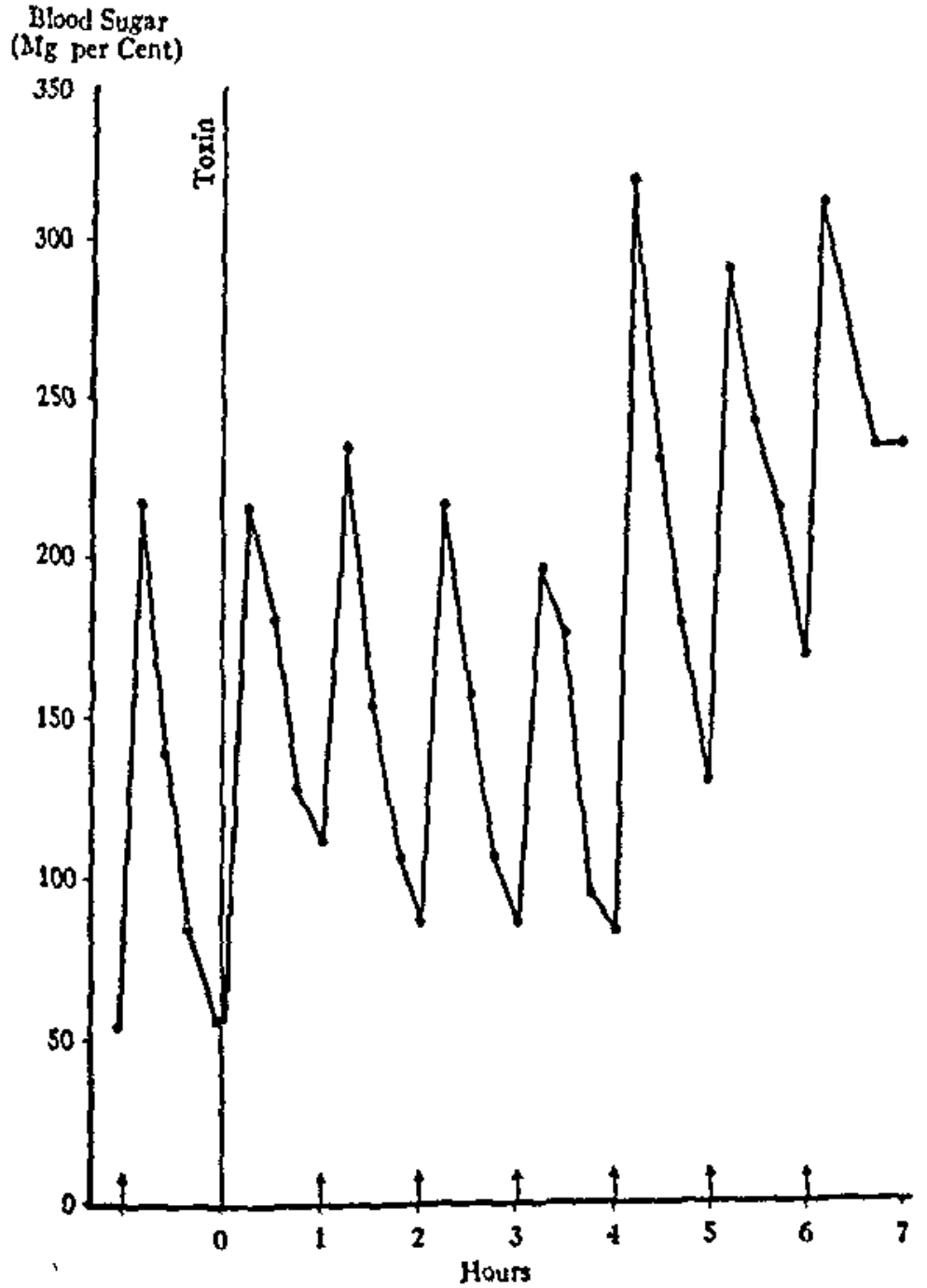
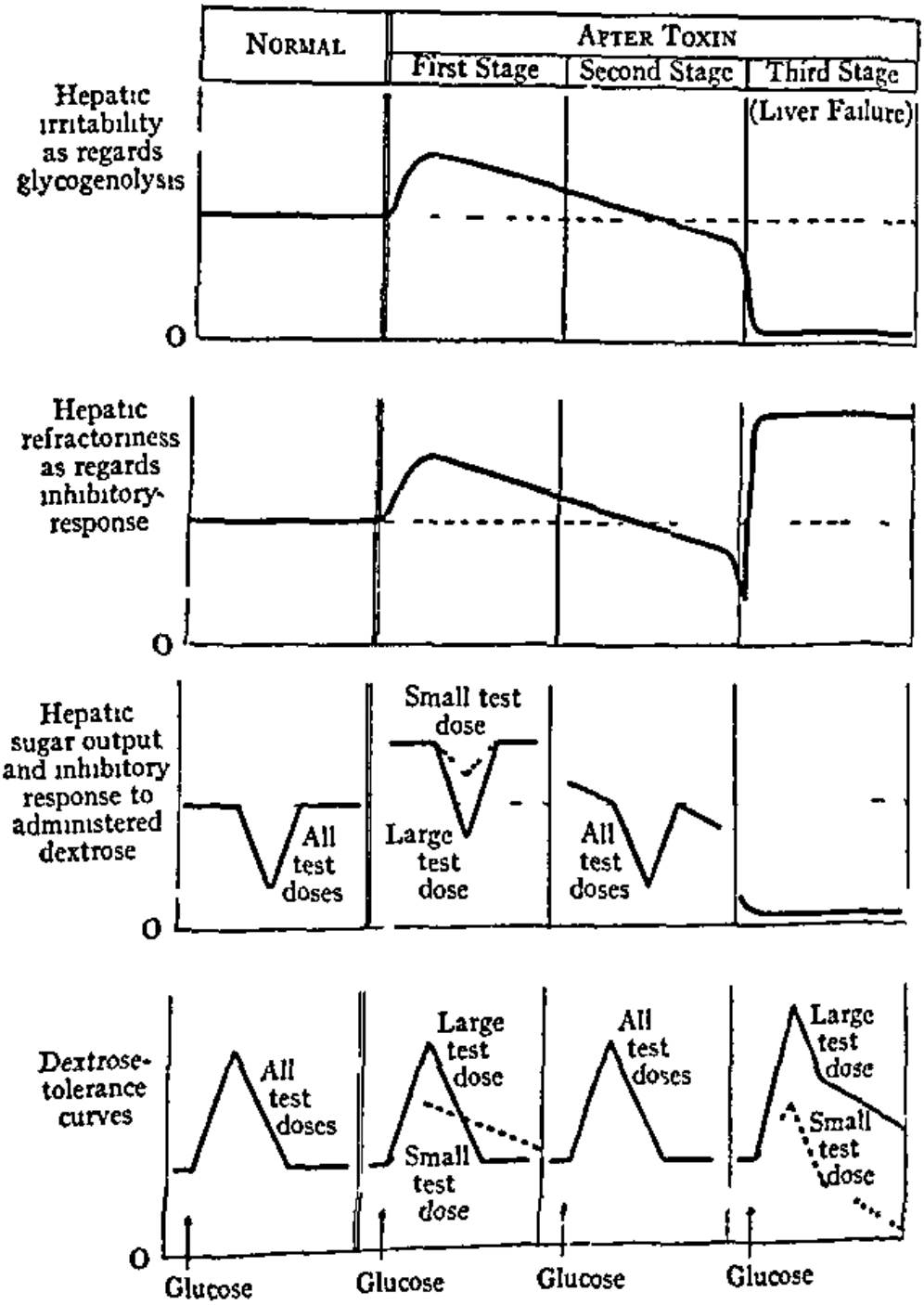


FIG 70.—Progressive toxemic liver damage Successive dextrose tolerance curves obtained with 0 9 m. of dextrose per kilogram of body weight administered intravenously Initial control curve is followed y toxin administration Arrows represent sugar administration (Soskin and Mirsky [70])


Normal
After Toxin
First Stage
Second Stage
Third Stage
(Liver Failure)
Hepatic irritability as regards glycogenolysis
0
Hepatic refractoriness as regards inhibitory response
0
Hepatic sugar output and inhibitory response to administered dextrose
All test doses
Small test dose
Large test dose
All test doses
0
Dextrose-tolerance curves
All test doses
Large test dose
Small test dose
All test doses
Large test dose
Small test dose
0
Glucose
Glucose
Glucose
Glucose

istered sugar, be great enough to overcome the refractory state of the organ, when a normal inhibitory response and therefore a normal tolerance curve may be obtained, (2) a return to the normal inhibitory reaction yielding apparently normal curves, and (3) a transitory phase of increased inhibition of glycogenolysis yielding supernormal curves, which passes rapidly into the stage of complete liver failure, with a cessation of sugar output and the reactions of the hepatectomized animal

From the practical standpoint it is noteworthy that a supposedly normal dextrose-tolerance curve may under appropriate circumstances represent a greater degree of liver damage than a 'diabetic' curve This probably accounts for the difficulty in correlating results of dextrose-tolerance tests with the clinical or pathological evidence of liver damage Such curves can be more correctly interpreted in the light of the cycle of events described above and in conjunction with other evidence as to the extent and duration of the hepatic impairment

It is evident that a series of dextrose tolerance tests performed at intervals during the course of a hepatic disorder can yield information of greater prognostic value than could possibly be derived from any single test It is also likely that a comparison of tolerance curves obtained with large and small doses of sugar might be of clinical value, since in stage 1 the large dose yields more normal curves than does the small dose, while in stage 3 the reverse is true In general stage 1 corresponds to the carbohydrate abnormalities observed in so-called 'hepatitis' (31, 32), while the disturbances described for stage 3 are seen in advanced hepatic cirrhosis (33 34)

Holmes (25) has reviewed the *in vitro* observations upon the effects of toxin on carbohydrate metabolism of liver The results of such work confirm the experimental and clinical observations detailed above The progressive effects demonstrated on liver slices and arranged in order of time sequence or of degrees of damage, are as follows first, an increased rate of glycogenolysis and a decreased ability to form glycogen from glucose (sugar can still be made from lactic and pyruvic acids and from alanine but cannot be stored) and second a decreased ability to convert the three carbon compounds into glucose and a more or less complete loss of the ability to form glycogen

THE INTRAVENOUS DEXTROSE TOLERANCE TEST FOR LIVER DYSFUNCTION

The important influence of the state of the liver on blood sugar regulation makes it desirable to be able to differentiate between hepatic and endocrine disturbances There have been a number of investigators who have reported that the oral dextrose tolerance curve is abnormal in liver disease, but no characteristics which would distinguish such a curve from that obtained in diabetes mellitus have ever been described (21, 24) Using a standardized intravenous procedure for the test, Soskin and his co-workers (35) have recently been able to obtain curves from

normal individuals, patients with known liver disease, and patients with mild diabetes mellitus, respectively, which are characteristic for each condition and which can be differentiated from each other The procedure which must be followed exactly if their standards are to be used, is as follows The test is done in the morning before breakfast One third gram of dextrose per kilogram of body weight, in a 50 per cent aqueous solution, is injected intravenously within a period of 3 5 minutes Blood samples are taken before the sugar administration and at ½, 1 and 2 hours thereafter These investigators used capillary blood obtained by

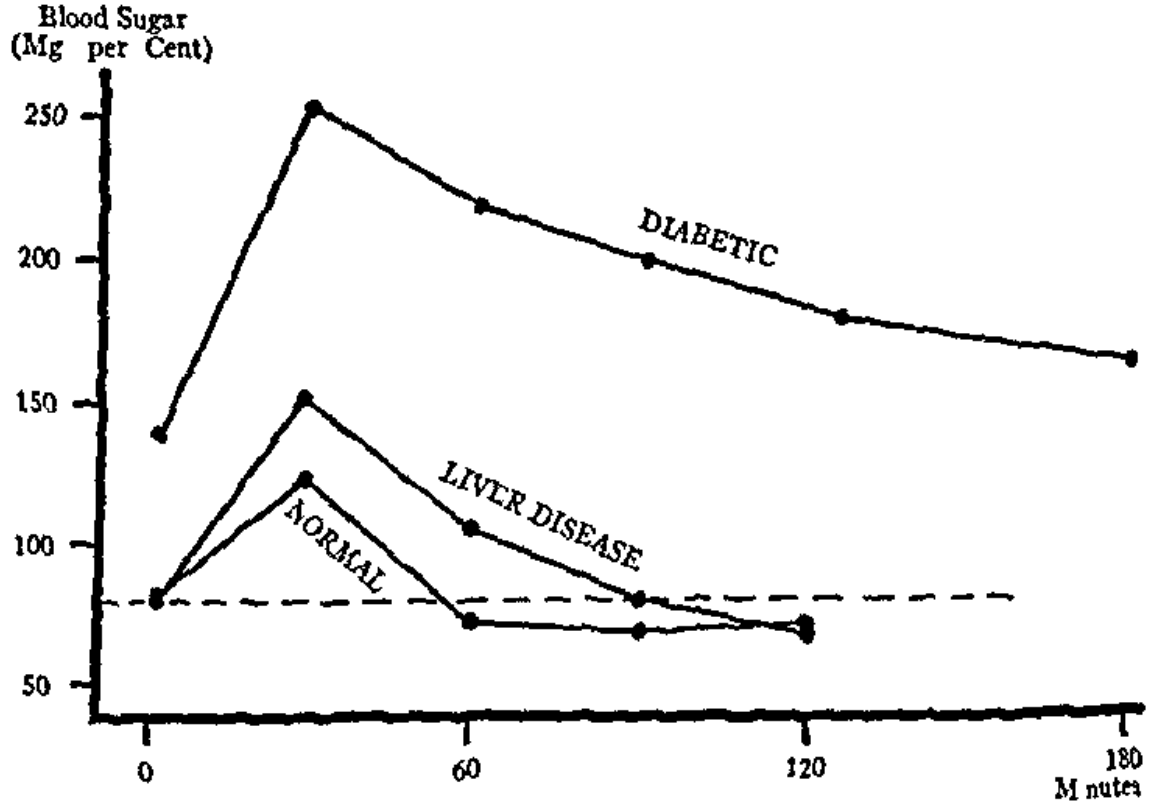


FIG 72 —The average intravenous-dextrose tolerance curves of 30 normal control individuals 25 of the mildest cases of diabetes mellitus that were available and 50 cases of proved mild or early liver disease The normal curve returns to the pre-injection level by 60 minutes the hepatic curve returns after 60 and before 120 minutes the diabetic curve returns after 120 minutes (Soskin [35])

finger puncture, and the micromodification of the Somogyi Shaffer Hartmann method for true blood sugar

Figure 72 shows the average curves for 30 normal control individuals 25 of the mildest cases of diabetes mellitus which were available (none had a fasting blood sugar level over 200 mg per cent and none required insulin for the control of their diabetes), and 50 cases of mild or early liver disease (clinically established and corroborated by several laboratory criteria) The wide spread between the three types of curve and the ease with which they can be differentiated is apparent As regards the variation between the individual tests which go to make up the aver

age curves not a single one of the 30 normal cases took as much as 60 minutes to return to the pre injection level This agrees with the normal standard previously reported by Tunbridge and Allibone (36) Not a single one of the 25 cases of mild diabetes took less than 120 minutes to return to the initial level Not a single one of the 50 cases of mild or early liver disease took as long as 120 minutes to return to the pre injection level although 13 of the 50 or approximately 25 per cent of these cases did cross the base line in less than 60 minutes

It might appear, at first glance from the average curves, that the differentiation between the diabetic and hepatic type can just as readily be made from the higher

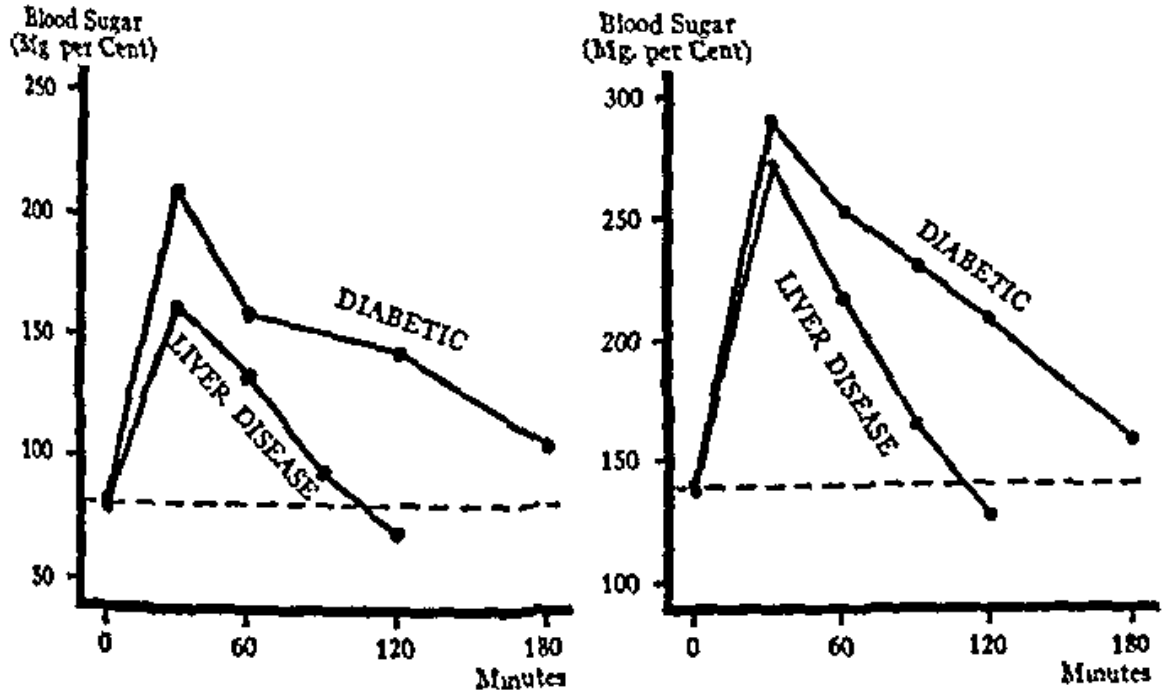


FIG 73 —Individual intravenous-dextrose tolerance curves in cases of diabetes and of liver disease which happened to start at identical fasting blood sugar levels The characteristic downslopes and the times of return to the pre injection levels are the criteria for differentiation (Soskin [35])

initial level and the higher peak value of the former This is not so when individual curves are considered The characteristics of the average curves depend upon the fact that more of the diabetic curves started at, and reached, higher levels However the range of these values in diabetes and in liver disease actually overlapped to some extent Figure 73 shows that, when this was the case, the characteristic downward slope of the curve and the time at which it crossed the base line were the real differentiating factors The curves in Figure 73 are for individual cases of diabetes and of liver disease, selected because they happened to start at identical fasting blood sugar levels It may be seen that, while the initial levels and highest peaks did not distinguish between the two conditions the characteristic down slope and time of return to the pre injection level permitted easy distinction

THERAPEUTIC USE OF HIGH CARBOHYDRATE DIETS IN LIVER DISEASE

Since Rosenbaum (37) in 1882 called attention to the depletion of hepatic glycogen following [illegible]

[illegible] damaged liver contains little glycogen (38) Concomitant with the loss of glycogen fatty changes appear in the liver after exposure to these hepatotoxic agents Rosenfeld (39) observed that animals fed carbohydrate are, in general less susceptible to any drug which produces accumulations of fat in the liver Furthermore, after such poisonings the feeding of dextrose aids recovery of the animal Since the early reports of Whipple and Sperry (40), Opie and Alford (41), and Graham (42) on the resistance to chloroform or phosphorus poisoning of animals fed large amounts of carbohydrate or animals with livers containing large stores of glycogen, there have been many similar observations (43) The protective action of a high carbohydrate intake has also been noted in the prevention of hepatic damage following experimental ligation of the common bile duct (44), operation for Eck fistula (45), partial hepatectomy (46), and experimental poisoning with the mushroom *Amanita phalloides* (47)

The various demonstrations of the lifesaving action of high carbohydrate intake on animals with experimentally damaged livers have been paralleled by clinical explorations of the therapeutic and prophylactic possibilities of administration of [illegible]

large amounts of carbohydrate The recent experimental results obtained by [illegible] man and his co workers have emphasized the therapeutic possibilities when ade [illegible] stuffs on liver disease, it is gen here has been some work which purports to show that high protein diets are as good or better than high carbohydrate diets There is good evidence that in certain specific types of poisoning, namely those due to selenium (53) and arsphenamine (54), protein is definitely superior to carbohydrate in protective value Indeed, in the exceptional case of sodium cyanide poisoning, high fat intake is better than either protein or carbohydrate (55) However, the evidence upon which the general superiority of protein is claimed is open to serious question An examination of the data of Ravdin and his co workers (56 57) reveals that most of their comparisons were made [illegible] protein content asis of comparison is, of course, adequate protein–high carbohydrate [illegible] gh protein–low-carbohydrate Table 38 summarizes such a comparison (made in the authors' laboratory) for carbon tetrachloride poisoning in rats It may be seen that the adequate protein–high carbohydrate diet was definitely superior in lifesaving

effect to both the high fat and the high protein diets Chemical examination showed a correspondingly higher glycogen content of those poisoned animals which had been on the high carbohydrate diet

It seems fair to conclude that except in those instances where protein seems to exert a specific action, its value depends upon its glycogenic and lipotropic properties Hence an adequate protein-high-carbohydrate diet is generally applicable In using such a diet, the increased requirements for the vitamins of the B complex should be satisfied And in this connection it is important to note that large amounts of carbohydrate, together with a high dosage of thiamine, would tend to produce fatty livers (58) unless counterbalanced by an adequate intake of choline or lipotropic amino acids

Even this brief survey of the subject points up the incompleteness of our present knowledge, especially as regards the particular effects of the various toxins en

TABLE 38

SUPERIORITY OF HIGH CARBOHYDRATE ADEQUATE PROTEIN DIET IN THE TREATMENT OF CARBON TETRACHLORIDE POISONING (MATTAR AND TAUBENHAUS [111])

TYPE OF DIET	NO OF RATS	SURVIVAL (DAYS)	LIVER (All Values in Gm per Cent)		
			Glycogen	Fat	Protein Nitrogen
High fat	12	12	0 58	10 86	2 50
High protein	12	17	1 67	5 68	2 34
High carbohydrate	12	28	2 27	6 06	2 66

countered clinically A systematic study of these and of the specific dietary combinations which are most effective in each case is certainly in order (59 60)

PHYSIOLOGIC BASIS OF INTRAVENOUS DEXTROSE THERAPY FOR DISEASES OF THE LIVER

On the basis of Rosenbaum's observations and Rosenfeld's theories Beddard (61) had suggested as early as 1908 that dextrose be used clinically in large quantities to restore the depleted reserves of hepatic glycogen in cases of delayed poisoning after chloroform anesthesia In addition to the administration of dextrose by mouth and by rectal enemas Beddard advised the intravenous use of a 6 per cent solution It is only recently, however, that the general introduction of adequate dextrose therapy for hepatic disease has been shown to produce a definite decrease in mortality In a series of cases in which acute hepatic insufficiency was treated with varying amounts of carbohydrate given by mouth and intravenously, Jones (51) found that in a group of 10 cases observed from 1922 to 1925, in which the

patients were given a diet low in fat and supposedly high in carbohydrate, the mortality was 90 per cent In only two instances was dextrose administered intravenously In the next five years, with diets somewhat higher in carbohydrates (300–400 gm daily) but with intravenous administration of dextrose in only four instances, there was 100 per cent mortality in 14 cases However, in the years 1930–35 when dextrose therapy was vigorous, 32 patients were treated with diets containing 400–500 gm of carbohydrate daily, 26 of them receiving dextrose intravenously, and the mortality was lowered to 63 per cent This author concluded "The more intensive the glucose therapy, the better the prognosis"

Despite these empiric observations, some difference of opinion still exists concerning the advantages of intravenous administration of dextrose if the patient can take the necessary dextrose or carbohydrate by mouth (62) But it should be pointed out that the *necessary* amount of carbohydrate is supplied by the amount of dextrose sufficient to raise the blood sugar to a level which will suppress the output of hepatic sugar Whereas the normal liver will respond to the usual postprandial hyperglycemia, the "irritable" liver in acute toxemia may require a much higher concentration of blood sugar to inhibit the formation of hepatic sugar That this is so was seen in the experiments previously described (p 270) in which there was a prompt response of the acutely poisoned liver in curtailing its output of sugar when large doses of dextrose were given intravenously, while small doses had little or no effect (29)

Furthermore as Cori and Cori (63) have pointed out concerning the normal liver, "the blood sugar concentration and not the amount of glucose administered must be regarded as important for the rate of glycogen deposition in the liver" Consequently, when an attempt is made to protect a damaged liver by means of deposition of glycogen therein, the blood sugar concentration may have to be raised to a level which it may not be possible to obtain by feeding carbohydrates In such cases intravenous infusion of dextrose is essential The fact that extreme hyperglycemia so produced may result in glycosuria should not deter one from such vigorous therapy As a matter of fact, this treatment has been successfully applied in diabetic patients with manifest or suspected liver disease (64)

Because of the glycosuria which may result from intravenous dextrose therapy, some physicians favor the routine use of insulin with the sugar However, it should be pointed out that, unless the patient is diabetic, the indiscriminate use of insulin may defeat the very purpose for which the dextrose is administered We have already referred to the evidence that, in the presence of sufficient insulin to maintain a normal constant blood sugar level, no additional insulin is necessary to obtain a normal hepatic response to administered sugar (65) Hence, the injection of insulin into a non diabetic person can produce no additional hepatic effect, although it does cause increased storage of glycogen in the muscles Bridge (66) has shown that the administration of a certain amount of sugar intravenously to nor

mal rabbits resulted in higher levels of liver glycogen when it was given by itself than when insulin was injected simultaneously This occurred despite the fact that the insulin caused no lowering of the blood sugar level When the proportions of administered sugar and insulin are such that a lowering of the blood sugar results the liver is actually stimulated to pour out more sugar and is deprived of glycogen rather than replenished with it Soskin Allweiss and Mirsky (29) have shown that the use of insulin with dextrose in the treatment of toxic non-diabetic animals shortens life animals receiving dextrose alone live longer

After prolonged intravenous injections of dextrose designed to suppress the sugar producing mechanism of the liver the organ requires an interval to recover from the inhibition of dextrose formation so that hypoglycemia may appear from 1 to 3 hours after the cessation of the infusion (67) This should be anticipated and treated with small doses of carbohydrate by mouth or intravenously if necessary

CLINICAL KETOSIS

Table 39 lists the abnormal physiological states and the clinical conditions in which ketosis is encountered It also indicates the particular causative factors involved in each instance As we have seen from the previous discussion in chapter x the fundamental disturbance underlying all ketosis is a relative or absolute lack of carbohydrate in the liver leading to an excessive breakdown of fat However the conditions leading to this fundamental disturbance can be divided into three subgroups according to the manner in which it is brought about namely (*a*) disturbances in food intake (*b*) impairment of liver function and (*c*) endocrine disorders It will be noted that there are a number of question marks in the table These are applied to certain of the endocrine mechanisms to indicate not only our fragmentary knowledge as to the way in which they operate but also our lack of complete assurance that they operate at all in a particular condition With these reservations however Table 39 completely relates clinical ketosis with our previous physiological considerations Certain key references to more detailed consideration of the several conditions are also included in the table

Von Gierke s disease and diabetes mellitus require some additional comment The former is exceptional in that it is the only condition in which ketosis is associated with large stores of glycogen in the liver But this glycogen is not available for use as is also evident from the fact that there is a low blood sugar level In Table 39 the glycogen in von Gierke s disease was therefore labeled abnormal In reality it is more likely that the glycogen itself does not differ from that found in normal livers but that the hepatic enzyme systems are abnormal with a consequent inability to mobilize the glycogen The net result as far as the organism is concerned is the same as if the glycogen were absent As regards diabetes mellitus it will be noted that the factor of insulin lack is designated relative or absolute This is because, unlike experimental pancreatic diabetes we still do not know

TABLE 39

CAUSATIVE FACTORS IN VARIOUS STATES OF KETOSIS (SOSKIN AND LEVINE [107])

	Clinical States	References	Deficient Carbohydrate Intake	Excessive Glycogenolysis	Disturbed Glycogenesis	Excess Demand for Carbohydrate	Abnormal Glycogen	Relative or Absolute Insulin Lack	Anterior Pituitary Excess	Adrenal Cortical Excess	Female Sex-Hormone Excess	Alkalosis	Dehydration
Disturbances in Food Intake	Starvation	(68, 69)	+						?				
	High fat diet	(70, 71)	+						?				
	Excessive vomiting	(72, 73)	+									+	
	Alkalosis	(74, 75, 76)										+	+
Impairment of Liver Function	Fever and infectious diseases	(77)	+	+	+	+							+
	Anesthesia	(78)		+	+								
	Hepatitis and early cirrhosis	(79, 80)		+	+								
	Advanced circulatory failure	(81)		+	+								
	Von Gierke's disease	(82, 83)					+						
Endocrine Disorders	Diabetes mellitus	(84, 85)		+	+			+	?	?			+
	Acromegaly	(86)						+	+				
	Adrenal cortical hyperfunction	(87)							?	+			
	Hyperthyroidism	(86)		+	+	+							+
	Pregnancy and menstruation	(88, 105)							?		+		
	Violent exercise	(106)				+							+

whether in human diabetes mellitus there is an actual deficiency of insulin or whether there is an excess of opposing endocrine factors From the practical therapeutic viewpoint, this, of course, makes little difference, since in either case the administration of exogenous insulin will *temporarily restore the disturbed endocrine balance*

Secondary effects of ketosis —It is not at all certain whether the occurrence of ketone bodies in the blood and urine is in itself harmful The evidence as to the *toxicity of acetoacetic acid is contradictory, to say the least (77)* Be that as it may, it is clear that the appearance of the ketones in excess of the amounts which can be metabolized by the peripheral tissues sets into motion a vicious cycle with a number of harmful secondary effects The fact that the ketones are organic acids necessitates their neutralization by sodium to preserve the normal pH range of the blood and to enable their excretion by the kidney The ketonuria is therefore accompanied by a loss from the body of fixed base and water Further loss of chloride results from the vomiting which often accompanies ketosis All these factors lead to dehydration and hemoconcentration, which, together with the loss of salts, result in an impairment of kidney function When this occurs the ability of the body to metabolize and otherwise deal with the ketoacids rapidly diminishes, and there begins a shift in the pH of the blood to an extent incompatible with consciousness and life

The post mortem findings, in individuals in whom ketosis was the predominating cause of death, support our analysis of the pathological physiology There are no specific organic lesions to be found There is a cerebral capillary dilatation, perivascular edema and acute degenerative changes in the cells of various parts of the central nervous system The findings in other parts of the body are those which are also seen in acute exsanguinating hemorrhage and in congestive heart failure In general, therefore, the tissue pathology might very well be accounted for by acidosis, dehydration, hemoconcentration and cerebral anoxia

The treatment of ketosis —For purposes of treatment, another classification of states of clinical ketosis may be made—namely, diabetes mellitus, on the one hand, and all other conditions on the other hand Diabetes is the only condition in which the original disturbance is a relative or absolute lack of insulin, *and in diabetes the most essential part of the treatment is the early, adequate, and persistent administration of insulin* This treatment will, of course, be rendered more effective by the simultaneous administration of adequate amounts of carbohydrate, water, and salt But the need for the hormone is paramount

It is equally important to remember that in non-diabetic ketosis the adminis

rdinal principle of the

er under conditions in

ary to accomplish this

purpose in the diabetic organism The non-diabetic organism already has an op-

tained until the simple clinical and laboratory evidences of ketosis, dehydration, hemoconcentration, and hypochloremia have been abolished

INSULIN RESISTANCE

In a number of clinical conditions the response of a patient to a given dose of insulin is less than that obtained in a normal individual Diabetic patients who were formerly well controlled by a small dose of insulin may, with the advent of one of those clinical states, be poorly controlled even with very large insulin dosages This phenomenon has been commonly referred to as "insulin resistance"

It is difficult to define normal insulin sensitivity very exactly, and there is no general agreement as to just how abnormal the response must be in extent and duration to be called "insulin resistance" Lawrence (89) has reserved the term for instances in which the etiology is unknown Strouse and his co workers (90) in their recent review of the subject chose to restrict their definition to cases of known or unknown etiology in which, after 48 hours' observation, 200 or more units of insulin could be administered without an appreciable lowering of the blood sugar

The various disturbances which might diminish the normal action of insulin may be listed as follows

1 Poor absorption from the subcutaneous tissues

4 mally to its endocrine regulators

5 Unusual antibody formation to insulin or to other proteins present in insulin preparations

Various clinical cases have been reported in the medical literature in which one or another of the above factors have been supposed to operate But there is little good evidence that the suspected factor was actually responsible, and our knowledge of mechanisms is incomplete and is derived partly from clinical observation and partly from animal experimentation

Root and his co workers (91) followed insulin absorption from the subcutaneous tissue by preparing a compound of insulin with radioactive iodine This compound did not differ from insulin in its physiological activity, and the quantity present in an area in which it had been injected could be estimated *from the degree* of radioactivity They found that their insulin compound was absorbed much more slowly from the subcutaneous tissues of diabetic patients manifesting insulin resistance than from the skin of other diabetic patients The absorptive factor in the insulin resistant cases was confirmed by the fact that they responded smartly to insulin administered by the intravenous route

serum and insulin This has usually been interpreted as indicating the presence of an anti insulin factor in the blood of insulin resistant individuals Such a substance might be an antibody of some sort, or the effect might be non specific and be due to an abnormally rapid rate of destruction of the added insulin The possibility of hormonal antagonists is supported by the experimental evidence discussed in chapter xxi and by the clinical observations of increased requirement for insulin by diabetic patients coincidentally with the onset of thyroid or pituitary manifestations As regards the formation of antibodies to insulin such cases occur but are rare (94 95) However, it has been observed that the insulin requirement of diabetics is likely to increase during the course of any allergic manifestations even though the patient is not allergic to insulin itself The reported cases of insulin resistance in which an insulin antagonist in the blood has apparently been demon

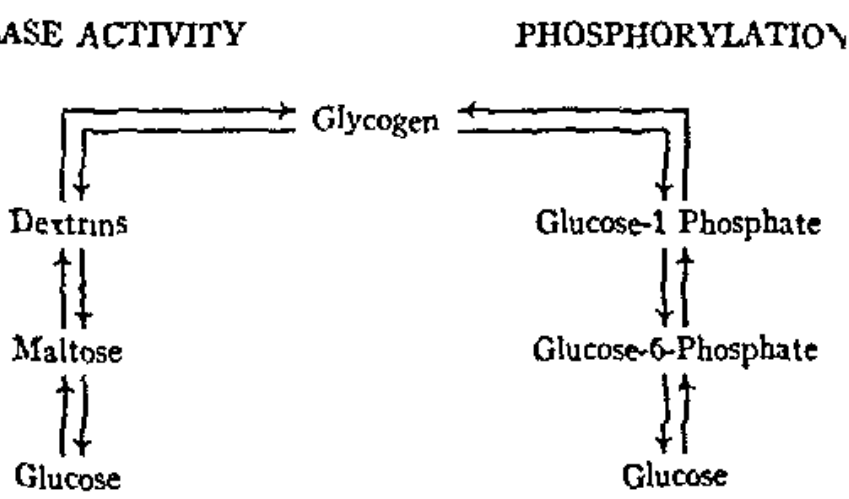


FIG 74.—Intermediary substances depending upon the mode of glycogenolysis. (Taubenhaus and Sokin [99])

strated are not accompanied by the type of evidence which would permit a determination of the nature of the antagonist involved

Insulin resistance is most commonly encountered in infections and febrile states The decreased effect of insulin has been variously ascribed to an overactivity of the [illegible] pos fun substantiated (21 29 96)

It was formerly thought that hepatic glycogenolysis normally occurred through amylase activity the glycogen being degraded through dextrins to maltose and to glucose (Fig 74) However Lee and Richter (97) who recently summarized the previous work on liver amylase and reported their own thorough studies on the subject pointed out that (*a*) even the highest amylase activity found in the blood liver and other organs is only of the order of 1/10 000 of the amylase activity of

PHYSIOLOGIC ACTION OF INSULIN IN SHOCK THERAPY OF THE PSYCHOSES

Schizophrenia and other psychoses have been treated with some degree of success by various forms of shock therapy including the induction of profound insulin hypoglycemia This influence of insulin has been attributed by some authors to a beneficial action of insulin upon the metabolism of the brain This interpretation is not warranted

The relationship between the blood sugar level and the utilization of carbohydrate by skeletal muscle was discussed in chapter xiv A similar relation between the blood sugar level and utilization of sugar has also been shown to hold for nerve and brain tissues in dogs and in man (100 101 102) It will be recalled that the lower plateau in the S shaped curve which expresses the relation of the blood sugar level to utilization of sugar indicates that the latter cannot be depressed below a certain minimal rate by any degree of hypoglycemia (chap xiv p 151) Marked hypoglycemias may therefore drive the available supply of sugar from the blood below the minimal requirements of the tissues Under such circumstances the muscle may have recourse to its stored glycogen or may perhaps turn to protein or fat as a source of energy It is generally agreed however that nerve tissue has little stored carbohydrate and cannot utilize protein or fat It follows that the nerve tissues during marked hypoglycemia are unable to maintain even the minimal rate of metabolism compatible with their well being This explains the recent reports that prolonged insulin hypoglycemia has led to irreversible damage to the central nervous system in experimental animals (103) and to similar pathologic changes and mental deterioration in schizophrenic patients (104) It may be concluded that insulin shock therapy has been well named!

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CHAPTER XXIII

COMPARATIVE PHYSIOLOGY OF DIABETES

IT WAS fortunate for the development of the science of metabolism that Mering and Minkowski, in 1890, chose to depancreatize dogs In this species pancreatic diabetes develops acutely and is characterized by hyperglycemia, glycosuria, polyuria, polydypsia, ketosis etc It bears a striking resemblance to diabetes mellitus in man even though the syndromes diverge in several details The effect of Mering and Minkowski s work on the dog was to advance our knowledge of carbohydrate metabolism rapidly by providing a very good experimental preparation

As early as 1879, Langendorf (1) had removed the pancreas of chickens and pigeons The operated birds did not exhibit glycosuria and they apparently died in extreme emaciation consequent to a loss of appetite The clear relationship between pancreatic function and normal carbohydrate metabolism could not have been deduced from this work with domesticated birds In 1891 Minkowski (2) confirmed the observations of Langendorf and extended his studies on the effects of pancreatectomy to several other species Since that time there have appeared sporadic studies in comparative diabetes, notably from the laboratories of Ivy (3, 4, 5, 6), Lukens (7, 8), Mirsky (9 10 11), and Houssay (12, 13)

Table 42 summarizes some of the characteristics of the syndromes which follow pancreatectomy in the various species which have been studied Diabetes mellitus in man is included for comparison It can be seen that the effects of the removal of the gland upon blood sugar, sugar excretion, protein breakdown, ketosis, and time of survival vary widely but not in any obviously related fashion Thus in both the dog and the cat the diabetic state is severe, as judged by glycosuria and protein breakdown, but while ketosis in the cat is very severe it is generally mild in the dog The depancreatized pig and goat, on the other hand, exhibit mild hyperglycemia and glycosuria, with little, if any, increase of protein breakdown above the normal rate The goat has a correspondingly mild ketonuria But the diabetic pig develops a very high level of ketone bodies in the blood which, however, does not seem to exert any effect on the acid base balance The duck and chicken develop glycosuria after pancreatectomy only occasionally and transiently However, removal of the gland in these birds produces anorexia, leading to death in marasmus The depanc
glycosuria but no ketosis, and survival without insulin
The mechanisms responsible for these species diff

TABLE 42

SPECIES VARIATION IN THE EFFECTS OF PANCREATECTOMY

SPECIES	BLOOD SUGAR (MG PER CENT)		URINE EXCRETION (GM/KG/DAY)			SURVIVAL (DAYS)	REMARKS	REFERENCES
	Normal	*Diabetic*	*Sugar*	*Nitrogen*	*Ketones*			
Man	60–90	341					ment, 27 units per day	(22)
	60–90	300–410					ment 20–50 units per day	(23)
Monkey	60–100	300–700	2 0–3 0	0 7 1 0	++++		0–200 mg per cent	(10)
Dog	50–70	310–345	2 8	1 0	++	10	able and transient	(24)
Cat		*212 788*	*3 2*	1 4	0 133	5		(24)
Rabbit	90–120	400–500	*Ca* 12 0		0	*38–120*	- ncreatectomy	(16)
Rat	102	190–233			++		y incomplete	(25)
Pig	*86–110*	*30–232*	0 2	0 5	0 179	9	but *no* acidosis	(7)
Goat		58–194	0 1	0 4	0 010	44	sis	(8)
Duck	95 126	100–200				41 163	emia is transient	(3)
	Ca 100	100				Prolonged	le to ketosis than normal	(9)
Chicken	200–439	140–880	+				for 1 week. Later birds	(5)
Owl	200–350	350–1 200					ition (due to anorexia)	
Toad	68	199				6	e Ketonemia *30–120 mg*	(11)
			(1 3 2 6%)			(30+ hours)	ival period is due to opera	(13)
Sculpin	7–48	113–490					- nce with liver and gastro ct	
Dogfish	159	402					te from pancreas in teleost	(26)
								(27)
	Diabetes Mellitus							
Man	60–90	200–600	0 2–2 0	0 13–0 38	++++			(28)

dated to date However there are indications that several different factors may play a role in modifying the diabetes of the various animals On the basis of the opposing activities of insulin on the one hand and the hormones of the anterior hypophysis the adrenal cortex and the thyroid on the other it might be supposed that the mild diabetes of some species may result from a characteristic or inbred weakness of the endocrine opponents of the pancreas This seems to be true for the pig which exhibits a diabetes similar in its characteristics to that of the hypophysectomized-depancreatized or adrenalectomized-depancreatized dog or cat The administration of anterior pituitary extracts intensifies the diabetic state of the p g (7) However the hypothesis of variable endocrine balance does not account for the modification of diabetes seen in other species Thus pituitary hormones do not induce manifest diabetes in the depancreatized duck (9)

Species differences after pancreatectomy might also be due to variations in the relative importance of factors other than insulin removed with the pancreas—for example the lipotropic function of pancreatic secretions This seems to be true in the case of monkeys Coll p (14) and Fulton (15) both reported that pancreatectomy in the monkey results in a mild diabetes resembling that of the Houssay dog However Mirsky (10) who maintained his depancreatized monkeys on a diet supplemented with pancreatin found a severe d abetic state resembling that of the cat and the ketosis was even more intense But the same investigator showed that the absence of lipotropic factors could not explain the failure of the duck to develop diabetes The inclusion of pancreatin in the diet of depancreatized ducks prolonged their survival time and prevented the intense weight loss but did not lead to hyperglycemia or glycosuria (9) The α-cells of the islands of Langerhans which in pancreatectomy are removed along with the insulin producing β-cells may also play an as yet undiscovered role in influencing the diabetic syndrome Alloxanized dogs in which the α-cells are undisturbed exhibit more intense glycosuria less ketosis and longer survival without insulin treatment than do depancreatized dogs (19)

It may well be that the apparent variation in the diabetic syndrome of a particular species may be due to the incomplete removal of insulin producing tissue Depancreatized rabbits have a prolonged survival time and little ketosis (16) while some alloxanized rabbits (in which presumably all β-cells are destroyed) exhibit a severe acidosis with a ketonemia of 120 mg per cent (17) Alloxanized rats (18) show no striking differences from depancreatized rats

On the basis of his observations Minkowski (2) made the generalization that carnivorous animals suffer from a more intense pancreatic diabetes than do the Herbivora. He and Weintraud (20) showed that unlike chickens p geons and ducks the carnivorous owls and hawks exhibit immediate glycosuria after pancreatectomy Mirsky (11) confirmed and extended the work on owls and he also attempted to change the response of the duck by a preliminary period of meat

feeding No conclusive results were obtained, although some of the meat fed ducks did develop a certain degree of hyperglycemia and glycosuria The previous dietary habits of an animal might influence the characteristics of its pancreatic diabetes by affecting the secretory activity of certain endocrine glands or by setting the metabolic reactions in the liver in one or another direction In the latter connection it might be well to recall the observation of F G Young (21) that the feeding of meat or non protein extracts of meat increases the severity of ketosis in dogs with metahypophyseal diabetes

Whatever the causes of species difference in diabetes may prove to be, the subject is by no means one of academic interest only It has already been pointed out that the etiology of diabetes mellitus in the human is unknown and that in the majority of cases it is evidently not due to pancreatic pathology (chap xxii, p 265) It may well be that further and more exact knowledge of the causes of species variation in the diabetic syndrome could suggest possible etiologic factors in man For this purpose, further work comparing alloxanized animals and studies on the gluconeogenetic response of various species to phlorhizin should be profitable

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CHAPTER XXIV

PRESENT FRONTIERS OF RESEARCH IN METABOLISM

ALTHOUGH this volume has dealt primarily with the metabolism of carbohydrate, it has been necessary to consider the metabolism of protein and fat to a considerable extent As a matter of fact, the division of the subject of metabolism into three compartments, related to the three major foodstuffs, is largely artificial, depending upon the limitations of the authors rather than upon any real separation of the subject matter In the light of more recent knowledge of intermediary metabolism, it seems likely that we shall soon cease to distinguish between the metabolisms of the different foodstuffs, once they have gone beyond certain stages, for, eventually, all of them give rise to very similar intermediary products, namely, the α and β ketoacids

INTERRELATIONSHIPS BETWEEN CARBOHYDRATE PROTEIN, AND FAT METABOLISM

Figure 18 (p 54) presents a composite scheme of the main pathways connecting the metabolism of carbohydrate, protein, and fat The supporting evidence is drawn from *in vivo*, perfusion, and *in vitro* experiments on different animals and under different conditions No single animal, organ, or type of tissue has been shown to be capable of performing all the reactions in the scheme Indeed, there is evidence that certain tissues lack the ability to carry on many of them The scheme therefore applies to the organism as a whole, i e , a certain tissue may carry the degradation of a foodstuff or the synthesis of an intermediate product to a given point and then pass on its end product, by way of the blood, to another tissue which completes the process

If the tentative scheme shown in Figure 18 is substantiated by future work, it will be possible to speak of a "final common pathway" for all the foodstuffs The intermediary metabolites composing the tricarboxylic acid cycle (see chap III) will then be regarded as a metabolic pool to which all the foodstuffs contribute and from which they can be regenerated (amination, CO_2 fixation) This will obviate much of the former discussion as to the interconvertibility of a particular foodstuff into another, for it will be realized that none of them are interconvertible in the sense that the constituent atoms of one pass directly and in a body into the other, while all of them are interconvertible, in the sense that the augmentation of the pool by a certain amount of intermediary material derived from any food

stuff may displace an equivalent amount of intermediary substance from the pool for the synthesis of another foodstuff

It might be objected that if the interchangeability of foodstuffs were as complete as is indicated by the scheme it should be possible to maintain adequate nutrition on a diet composed solely of any one of the foodstuffs But we know that only protein—and indeed only certain proteins—can be used in this way and then for limited periods of time only The answer to this objection lies not in any lack of interconvertibility but in the fact that animal metabolism is incomplete Animals cannot synthesize certain essential food materials but must obtain them from plant and mineral sources These essential accessory food factors comprise (1) the essential amino acids (2) the essential fatty acids (3) the vitamins and (4) the minerals It happens that only a mixed dietary of natural foods will contain the necessary amounts of all the accessory food factors

SIGNIFICANCE OF *in vitro* RESULTS

The best available scheme for the dynamics of carbohydrate metabolism was presented in chapter III But it must be emphasized that despite its general plausibility and inner logic it is only a tentative outline The data for it are derived from work done with intact with eviscerated and with hepatectomized animals and from observations made after the removal of various endocrine glands etc The preparations used for *in vitro* work include organ slices minced tissues and enzyme extracts

The various techniques of *in vitro* work have been invaluable for the development of our present concepts of intermediary metabolism but they suffer from several inherent limitations which are not always appreciated or emphasized Even tissue slices in which there is presumably the least physical damage to individual cells do not exhibit quite the same metabolic behavior as do the parent tissues *in vivo* For example liver slices cannot be induced to deposit glycogen (except rarely and to an insignificant degree) (1 2 3) as the organ so readily does *in vivo* The liver slice *in vitro* appears to be exclusively in the phase of glycogenolysis In this connection it may be pertinent to consider the fact that the intact liver possesses a dual blood supply each supply differing in rate of flow pressure and oxygen and CO_2 contents The cells of the liver slice *in vitro* must function in a uniform medium Turning from liver to brain we note that the highest *in vitro* oxygen consumption of cortical slices is only from one third to one half the oxygen consumption of whole brain *in vivo* (4 5 6) Obviously some unknown factors modify metabolism when tissues are separated from their normal environments

Mincing of tissues introduces even more serious deviations For example while an intact thin muscle (diaphragm or abdominal muscle) retains its ability to deposit glycogen from glucose (7 8 9) and can also use the glucose in the medium

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Mincing of tissues introduces even more serious deviations For example, while an intact thin muscle (diaphragm or abdominal muscle) retains its ability to deposit glycogen from glucose (7, 8, 9) and can also use the glucose in the medium

for energy purposes, mincing interferes with the entry of glucose into the cells for either purpose

Cell free extracts are a step further removed from normal relationships The generally used muscle extract of Meyerhof (10) contains the stable systems soluble in 0 6 per cent potassium chloride or water The water insoluble enzyme proteins, such as myosin, are not present, and the creatine phosphate hydrolyzes during the preparation of the extract It is obvious that the carbohydrate metabolism of such an extract is quantitatively and qualitatively different from that of intact muscle For example, it is well known that many tissues (e g , muscle) show a greater breakdown of carbohydrate and a larger formation of lactic acid during anoxia than during adequate oxygenation The inhibitory influence of oxygen on the rate of glycolysis is known as the "Pasteur effect" (11, 12) The exact mechanism of this effect in intact tissues is not entirely clear Among the factors which may be involved are (*a*) the breakdown of organic phosphate during anoxia, providing excess inorganic phosphate, which would orient the reactions toward glycolysis (13), and (*b*) the fact that many enzyme proteins involved in glycolysis are active in the –SH state (reduced) and may therefore be inhibited by an increased oxygen tension (14, 15) Whatever its mechanism, the Pasteur effect is an important regulatory phenomenon in carbohydrate metabolism *in vivo*—a mechanism which is completely lacking in tissue extracts

From even these few considerations it becomes obvious that extreme caution is necessary in applying *in vitro* data to the elucidation of *in vivo* metabolism Furthermore, a homogeneous cell free enzyme extract, even if it contained all the cell proteins in their *in vivo* proportions, would not be very comparable to the living cell In the latter, heterogeneity and structural separation, etc , make it possible to have a number of zones within a single cell, each differing as to pH, mineral composition, etc , and each varying in metabolic activity External influences, both physical and chemical, may therefore influence the metabolism of the cell by inducing changes in its internal structure For example, the structural change induced in myosin by the nerve impulse activates carbohydrate breakdown and alters the rate of oxygen consumption The rate of metabolism is also influenced in unknown fashion by thyroid hormone or by dinitrophenol These substances may act by bringing together links in the respiratory chain which, although always present in the cell, are usually separated from each other in some way

More specifically, Stannard (16, 17), Korr (18), and others (19, 20) have shown that, in certain tissues, work or chemical stimulation not only raises the rate of oxygen consumption but alters the pathway by which it is used The low oxygen consumption of these tissues at rest is resistant to the influence of cyanide despite the presence of the cytochrome system on which the poison acts When the tissues are stimulated, the oxygen consumption rises and becomes sensitive to cyanide

Apparently the stimulus in some way links the idle cytochrome system to the de
hydrogenases Similarly, the work of Sacks (21, 22, 23) and of Flock and Bollman
(24 25) indicates that the scheme of phosphorylations via adenosine triphosphate
(ATP), outlined in chapter IV, may apply to muscle at rest but may not be wholly
valid for the same tissue during work Although this work has been criticized
(26, 27), it should put us on guard against regarding the presently accepted meta
bolic schemes as either complete or final

In addition, it should again be recalled that the scheme of intermediary carbo
hydrate metabolism has been constructed from data obtained in different animals
and tissues It is a composite picture, and not every tissue or organ conforms to it
Thus the liver produces very little lactic acid, and yet it can build up hexoses and
glycogen from lactate (28) For the liver therefore the scheme requires modifica
tion to account for these phenomena To cite another example, skeletal muscle
tissue requires insulin for good rates of glycogen synthesis from glucose (7, 29) The
heart and kidney, on the other hand, may deposit greater than normal amounts
of glycogen when the blood sugar level is high but insulin is absent (30 31)

Taking into account all the pitfalls inherent in the various *in vitro* technics, we
may sum up by stating that, when a reaction or a series of reactions is shown to
proceed *in vitro*, we can conclude that these same reactions can, but do not neces
sarily, occur in the living intact organism A negative *in vitro* result is wholly in-
conclusive, since it may simply depend upon the conditions of the experiment All
in vitro data must eventually be checked *in vivo*, in order to acquire serious sig
nificance in our concepts of normal metabolism For this purpose, the labeled
molecule technic (radioactive or isotopic) has already proved its usefulness (32
33) The intravital staining technique of Gomori and others (34 35) and spectro
photometry of living tissues (36, 37, 38) also hold promise for the future

THE NATURE OF HORMONE ACTION

The previous discussions concerning the action of insulin (chap XVI p 180)
made it clear that glycogen deposition from glucose could proceed at a relatively
slow rate in the complete absence of the hormone Apparently the enzyme sys
tems necessary for the polymerization of glucose are present in the completely de
pancreatized animal, but the rates of their activity can be markedly enhanced by
insulin The hormone is, therefore not a necessary cell enzyme itself but a regu
lator of rates of reaction This point of view is supported by a consideration of the
amount of insulin which must be administered to restore the normal metabolic
state in a depancreatized animal This has been shown to be of the order of 230γ
for a 10-kg dog per day Even if no destruction of the administered insulin oc
curred, this would result in a concentration of approximately 0 3γ per 100 gm of
tissue water This order of magnitude is far lower than that of the concentration

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